

NO MAN'S PARADISE:
LEAD BURDEN AND
DIET RECONSTRUCTION FROM
HUMAN SKELETAL REMAINS
IN A COLONIAL CEMETERY
FROM ANTIGUA

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Abstract

The primary focus of this thesis is to examine the relationship between diet, as reconstructed via stable isotope analysis, and bone lead levels, quantified by trace element analysis for individuals interred at the Royal Naval Hospital Cemetery (RNHC), A.D. 1793-1822, in Antigua, West Indies. Individuals of both African and European ancestries were recovered from this colonial-era cemetery, and samples from their remains were analyzed to determine stable carbon and nitrogen isotope values (as a proxy for diet), and bone lead levels. The data were then compared in order to elucidate any association among the variables. This investigation revealed that the relationship between diet and lead may have been affected by many variables including ancestry, status, agency, and duration of stay in the West Indies. However, from the results presented in this thesis, the strongest correlation between stable isotope signatures and bone lead levels is in the relationship between $\delta^{13}\text{C}_{\text{collagen}}$ and lead for individuals consuming a diet primarily consisting of C_3 staple starches and C_3 fed animals.

A secondary focus of this thesis is to estimate the extent to which the individuals interred at the RNHC may have suffered from symptoms of lead poisoning. Through conversion of bone lead levels to blood lead levels, potential symptomatology may be estimated in order to determine the percentage of individuals from the population that may have experienced mild to severe lead poisoning. In this population, a majority of individuals had high enough blood lead levels that they may have suffered from a range of symptoms associated with exposure to lead, which is not inconsistent with historical assertions that lead poisoning was of considerable detriment to the health and well-being of individuals serving in the British military in the colonial Caribbean. This study provides further insight into the health and lifeways of lower-ranking naval personnel and enslaved labourers owned by the Navy in the late eighteenth and early nineteenth century West Indies.

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Chapter 1: Introduction

1.1 Thesis Objectives

The primary objective of this thesis is to examine the relationship between diet, as reconstructed through stable carbon (C) and nitrogen (N) isotope analysis of bone, and bone lead levels for individuals interred at the Royal Naval Hospital Cemetery (RNHC) (PAH-83) on the island of Antigua. The hospital and cemetery were in use from A.D. 1793–1822 (Varney and Nicholson 2001). A secondary objective is to study the potential negative health effects that lead exposure may have had on the individuals buried at the RNHC, by calculating blood lead levels from bone lead levels, in order to investigate the veracity of the historical claim that lead poisoning resulted in the demise of the British military in the West Indies (Buckley 1978).

The RNHC was a non-segregated burial ground for individuals of both European and African ancestries, making it a unique cemetery site within the West Indian colonial context (Varney 2003; Varney and Nicholson 2001). Stable carbon and nitrogen isotope analysis was carried out on bone samples from the individuals at the RNHC in order to determine differences in diet between the groups of distinct ancestral and socioeconomic backgrounds (Varney 2003, 2011). Although differences in diet were encountered between those of European and those of African descent (Varney 2003), no significant differences were distinguishable between these two groups with respect to bone lead levels (Giffin 2014, 2015).

Historical documentation indicates that lead was ubiquitous during the colonial period in the West Indies, found in foodstuffs, medicines, ceramic glazes and pewter, water catchments, rum distillation equipment, and sugar processing equipment (Buckley 1978; Eisinger 1982; Handler et al. 1986). Although it has been suggested that rum, a product of sugarcane, contaminated with lead may have been a major contributor to lead exposure in the colonial West Indies (Buckley 1978; Handler et al. 1986), there have been no studies that have attempted to determine whether the consumption of foodstuffs contaminated with lead, including rum and sugar, is associated with stable isotope signatures from human skeletal remains. Stable isotope analysis of human remains has generally been applied to reconstruct the diet of past populations in terms of general food categories (Katzenberg 2008). This thesis investigates the relationship between stable isotopes, as a proxy for diet, and lead exposure to determine whether lead levels

in bone are reflective of adherence to a particular dietary regime, and whether this relates to an individual's ancestry and/or status.

It has been hypothesized that lead poisoning may have been a contributing factor to the failure of the British military in the Caribbean (Buckley 1978). Although bone lead levels alone cannot reveal whether an individual suffered symptoms of lead poisoning, blood lead levels have been estimated using bone lead levels in past studies in order to assess potential negative health effects of lead exposure (Corruccini et al. 1987; Keenleyside et al. 1997; Kjaer et al. 2009). Many limitations are associated with the conversion of bone to blood lead levels, given that there is no simple relationship between the two (Ahlgren et al. 1980). These limitations are discussed in depth in Chapter 6. Though these are rough approximations of blood lead levels, these calculations may be used to determine what percentage of the cemetery population was most likely to have suffered from the ill effects of lead poisoning. The lack of a statistically significant difference in bone lead levels between those of African and those of European descent (Giffin 2014, 2015) is suggestive that there was no differential access to lead contaminated goods or occupational exposure based on ancestry. However, a comparison between blood lead levels between the two ancestral groups is carried out in order to determine if there was a noteworthy difference in lead related health problems based on ancestry.

This research contributes new insight into the extent to which lead exposure may have specifically affected the individuals buried at the RNHC, as well as colonial era Naval personnel and enslaved labourers owned by the Navy in general. It also indicates whether those of European and African ancestry may have suffered from the ill effects of lead poisoning, and whether this was different based on ancestry, providing evidence for the social determinants of health in colonial Antigua. Finally, this research combines historical and bioarchaeological evidence to further elucidate the lifeways of lower-ranking naval personnel, both free and enslaved, in the West Indies.

1.2 Hypotheses to be Tested

The aim of this research is to answer the following questions regarding the relationship between diet and bone lead levels, as well as blood lead levels and symptoms of lead poisoning for the population recovered from the RNHC:

1. Is there a correlation between diet and lead exposure, as indicated by stable isotope signatures and bone lead levels?

2. If a correlation exists, what dietary patterns are associated with increased and decreased bone lead levels?
3. Do approximate blood lead levels calculated for the individuals interred at the RNHC demonstrate that at least a portion of the population may have suffered from lead poisoning during life? If so, is the assertion that lead poisoning was a major contributor to the downfall of the British military in the West Indies valid?
4. Is there a significant difference in blood lead levels between those of African and European descent, and is lead exposure indicative of differential well-being or access to goods contaminated with lead based on ancestry and/or socioeconomic status?

An exploration of the relationship between stable carbon and nitrogen isotopes and lead concentrations in bone samples should demonstrate whether a correlation exists between diet and exposure to lead during life. Although stable carbon and nitrogen isotope signatures represent general food categories rather than specific foods, it should be possible to determine whether or not there is an association between lead and diet if lead levels coincide with particular dietary patterns. It is possible that both direct and indirect relationships exist between diet and bone lead levels. Direct associations would be reflective of the consumption of contaminated food products, such as rum, having an effect on stable isotope signatures and an effect on bone lead levels. Indirect relationships would reflect an association between higher or lower bone lead levels and the consumption of particular types of foods, without those foods themselves necessarily having been contaminated with lead. In the case of an indirect relationship, lead exposure could be the result of consumption of a contaminated food that is poorly visible in the stable isotope signatures, or from a non-comestible source of lead.

It can be expected that, if a direct relationship between lead and diet exists, it will be most prominently shown between lead levels and $\delta^{13}\text{C}$ signatures in bone apatite. It is specifically $\delta^{13}\text{C}$ signatures from bone apatite that seem likely to show a correlation with lead levels because the stable isotope signatures of apatite are demonstrative of the diet as a whole, rather than being preferential to dietary protein (i.e., $\delta^{13}\text{C}$ from bone collagen) or trophic level (i.e., $\delta^{15}\text{N}$ from bone collagen) (Ambrose and Norr 1993; DeNiro and Epstein 1981). Thus, since rum is suspected to be the most probable source of lead exposure via foodstuffs consumed (Buckley 1978; Handler et al. 1986), and rum is the product of a C_4 plant (sugarcane), it may be possible to see increases in lead that coincide with more positive $\delta^{13}\text{C}_{\text{apatite}}$ values. It has been

proposed that alcohol may affect stable carbon isotope signatures if consumed in substantial quantities (Katzenberg et al. 2000), which would have been the case for individuals serving in the Navy (Pack 1982). Rum does not contain carbohydrates in the form of sugars, therefore it is the alcohol itself that would contribute to stable isotope signatures. The $\delta^{13}\text{C}$ value of rum ranges from -11‰ to -14‰ (Katzenberg et al. 2000). Rum also does not contain any protein and therefore would contribute less substantially to the $\delta^{13}\text{C}_{\text{collagen}}$ values than other foods being consumed that are higher in protein. Ambrose and Norr (1993) suggested that, in a diet of 20% protein, energy sources would contribute 29–34% of the carbon in collagen. Although $\delta^{13}\text{C}_{\text{collagen}}$ preferentially reflects dietary protein, it also may be possible to see a correlation between bone lead levels and $\delta^{13}\text{C}_{\text{collagen}}$ signatures, as they can also indicate consumption of C_3 and/or C_4 plant products.

With regards to the association between $\delta^{15}\text{N}$ and bone lead levels, it can be expected that no particularly strong association will be found between these variables because $\delta^{15}\text{N}$ tends to reflect the trophic level of the diet, with higher nitrogen levels demonstrating the consumption of animal tissues, and very high nitrogen levels demonstrating the consumption of marine animal resources, such as fish (DeNiro and Epstein 1981; Schoeninger and DeNiro 1984). Stable nitrogen isotope values are also more influenced by dietary components that have high concentrations of nitrogen, and that are high in protein (Makarewicz and Sealy 2015; Phillips and Koch 2002). Since rum is a plant product, free of protein, and having only a negligible concentration of nitrogen (Delavante 2004), it would not have an impact on the $\delta^{15}\text{N}$ signature. Thus, this eliminates the potential for a direct relationship between $\delta^{15}\text{N}$ values and bone lead levels. Any association between these variables must therefore be an indirect relationship, and thus, a reflection of the exposure to lead for individuals consuming a diet with a particular quantity or type of protein.

Factors that may confound the interpretation of any correlations between lead levels and stable isotope signatures include: 1) foodstuffs contaminated with lead being indistinguishable in the stable isotope signatures; 2) lead exposure not being entirely the result of consumption of contaminated foodstuffs but also the result of occupational exposure, use of goods fabricated with lead, or medicinal use of lead; and 3) dietary regimes differing between ancestral groups. Nevertheless, diet is still not a perfect predictor of ancestry, and some individuals may consume

dissimilar foods to other individuals of the same ancestral groups. These concerns are taken into consideration in the comparison of lead levels with stable isotope signatures.

The estimation of clinical symptoms of lead poisoning may be made by converting bone lead levels to blood lead levels (Corruccini et al. 1987; Handler et al. 1986), as mentioned above. If the historical and archaeological sources are accurate in their assertions that lead poisoning was of considerable detriment to the health of those in the British military serving in the West Indies (Buckley 1978), as well as those enslaved in the West Indies (Handler et al. 1986), then there should be some individuals from the RNHC whose blood lead levels are high enough that they would have experienced negative health effects. Additionally, though there was no evidence of a significant difference in bone lead levels between those of African and those of European descent (Giffin 2014, 2015), there is potential for a significant difference in blood lead levels between individuals of different ancestries. This is because bone lead levels and blood lead levels are distinct measurements of lead in the body, with the former representing long-term accumulation of lead (Aufderheide 1989), and the latter, representing recent exposure to lead (Somervaille et al. 1988). When blood lead levels are estimated from bone lead levels they are an estimate of the average blood lead level for an individual for the duration of lead exposure (Somervaille et al. 1988). Age may be a confounding factor in the difference between bone lead levels in the two ancestral groups given that individuals of African descent tended to be older than those of European descent at death. Thus, blood lead levels calculated based on the age at death for the individuals at the RNHC may differ significantly between those of African and those of European descent.

1.3 Structure of the Thesis

Background information is provided in Chapter 2. This includes historical documentation of the RNHC, the naval dockyard in Antigua, and its inhabitants during the colonial period. An overview of diet, health, and lead poisoning in the colonial West Indies is also provided.

Chapter 3 comprises reviews of the basic principles of both stable isotope analysis and trace element analysis.

In Chapter 4, materials and methods are presented. This includes an overview of the excavations at the RNHC, the stable isotope analysis and quantitative lead analysis of individuals who were buried at the RNHC, as well as a brief description of the statistical analysis used to study this population.

Chapter 5 provides results for the tested hypotheses. Results for the correlation between diet and lead are presented, followed by results for the estimation of blood lead levels for the RNHC population.

Chapter 6 presents discussion of the results from Chapter 5, including interpretation of the relationship between diet and lead, as well as the likelihood that the RNHC population was affected by lead poisoning.

Conclusions and future avenues of investigation are presented in Chapter 7.

Chapter 2: Background Information

2.1 Introduction

This chapter provides background information pertinent to the time period (A.D. 1793–1822) and geographical location of the Royal Naval Hospital Cemetery (RNHC). This includes contextual information on the island of Antigua, focusing on the historical documentation of the Royal Naval Dockyard, as well as the inhabitants of Antigua and the dockyard during the colonial period. Background of an historical and archaeological nature is also presented for the Royal Naval Hospital. Finally, this chapter contains an overview of diet, health, and lead poisoning in the colonial West Indies.

2.2 Antigua, the Royal Naval Dockyard, the Inhabitants, and the Royal Naval Hospital

2.2.1 Antigua

Antigua is a small Caribbean island of 280 square kilometers, forming part of the Leeward Islands in the Lesser Antilles. The term Caribbean refers to the region including the Caribbean Sea and the lands surrounding it. The Greater Antilles are the larger islands to the north of the Caribbean Sea and include Cuba, Hispaniola, Puerto Rico, and Jamaica. The Lesser Antilles are the smaller islands to the east of the Caribbean Sea, in which Antigua is included. The West Indies typically refers to all the Antilles (Richardson 1992). A map of Antigua and the surrounding area is shown in Figure 2.1.

Antigua was officially recognized as an English colony in 1636 (Sheridan 1973). In general, the history of Antigua has been poorly documented, though it became the leading sugar producing island of British possession in the Lesser Antilles during the eighteenth century (Dunn 1972; Sheridan 1973). The colonial period was a tumultuous time in the Leeward Islands due to threat of raids by indigenous peoples, attacks by other European colonial powers, slave revolts, and internal factionalism (Dunn 1972; Parker 2011). Other major complications for early settlers were the lack of fresh water sources and droughts.

Early on in the colonial period, during the mid-seventeenth century, tobacco was the main crop grown in Antigua, along with limited quantities of cotton, ginger, indigo, and sugarcane (Sheridan 1973). At the time, farms were small and labour was provided primarily by white indentured servants (Dunn 1972), “white” referring to individuals of European ancestry.

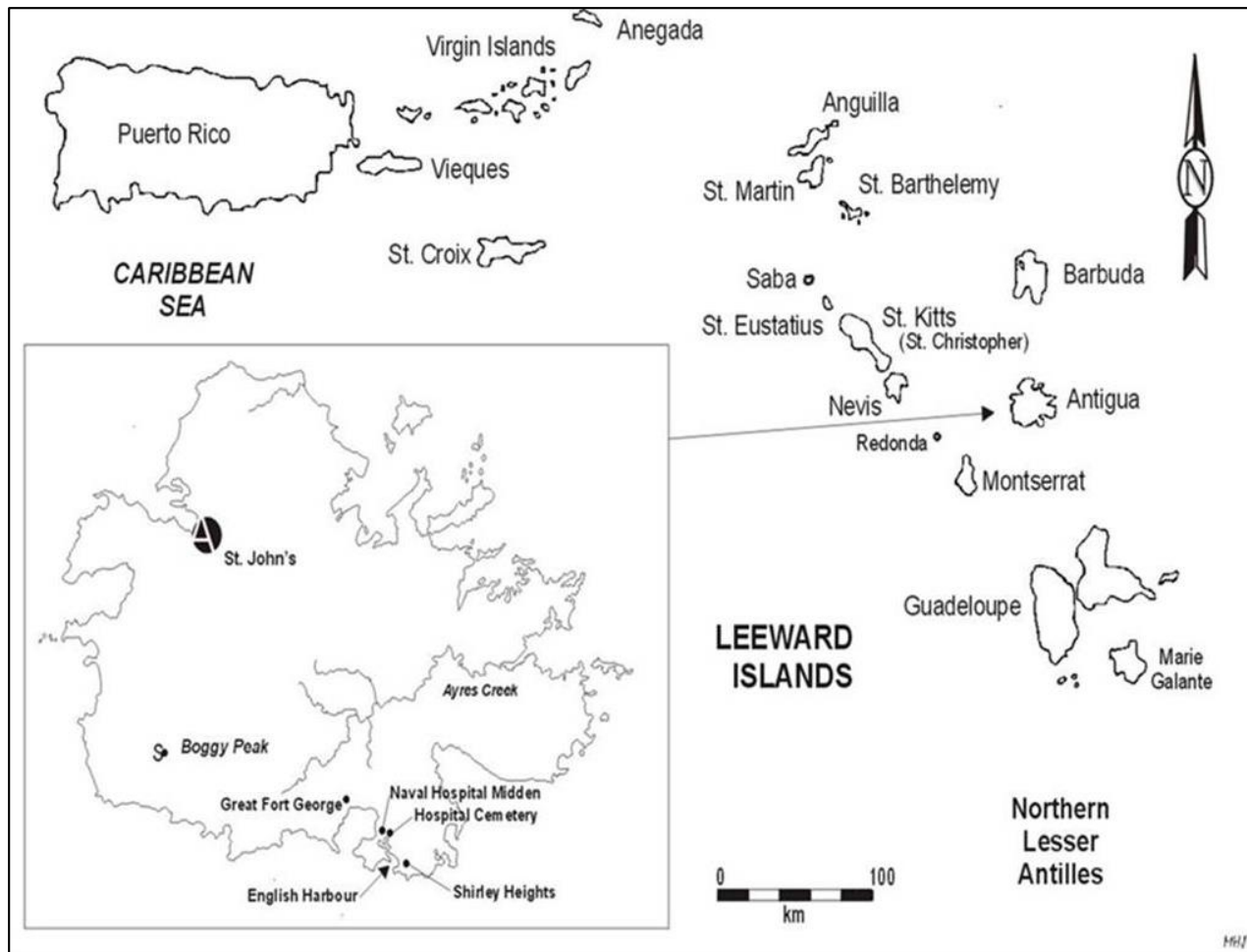


Figure 2.1 Map of the northern Lesser Antilles with Antigua inset. Map courtesy of Michael H.J. Turney.

The sugar economy grew substantially between 1680–1706, until it became the base of the Antiguan economy and a large portion of agricultural land was turned over to growing sugarcane, resulting in a near monoculture (Sheridan 1973). A much smaller proportion of land was used to raise livestock, such as sheep and cattle, and grow provision crops. Approximately 30% of cultivated lands in Antigua were for provision crops in the late eighteenth and early nineteenth centuries (Higman 1984). When agriculture shifted to sugarcane from tobacco, the small farms became large plantations, and white labour was replaced by the labour of enslaved peoples from Africa and their descendants born on the island (Dunn 1972). Antigua remained a British colony until it gained its independence from Britain in 1981 (Nicholson 1986).

2.2.2 The Royal Naval Dockyard

The geography of Antigua, with many sheltered bays and harbours, would become important in the colonial period for the defense of the island and development of the Royal Naval Dockyard at English Harbour. Construction of the dockyard began in 1725, though the harbour had been used since the 1670s as a hurricane shelter and to careen ships (Nicholson 1986; Sheridan 1973). The dockyard was used to maintain and repair ships belonging to the Royal Navy that had been sent to protect British possessions in the West Indies and to prevent Antiguan planters from trading with foreign ships (Nicholson 1986; Ross 1961). Given the proximity of the islands to one another in the Lesser Antilles, the unstable relations of the European colonial powers, and the competitive nature of the sugar industry, a military presence was necessary to prevent the destruction of property on Antigua by invading rivals (Weaver 2002).

Infrastructure at the dockyard was developed during the eighteenth century in order to maintain a permanent squadron at English Harbour (Weaver 2002). The harbour was deepened, the docks reinforced with copper, and water catchments were built to store rainwater in tanks for supplying the ships and the inhabitants of the dockyard (Anonymous 1844; Luffman 1789; Ross 1961). A visitor to the dockyard in the nineteenth century described the extensive buildings including storehouses, pay offices, naval officers' quarters, stores, working mast-houses, guard houses, the boat-house, the engineers' workshop, the commissioner's room, the blacksmith shop, the residences of the shipwright and the superintendent, storage for ordnance, privates' barracks, the hospital, and victualing offices (Anonymous 1844). A permanent military presence at the harbour was desired in order to both harass the French trade efforts and to protect against French raids. An additional task of the British military presence at English Harbour was to protect the white plantation owners from slave uprisings (Weaver 2002). The dockyard, well-secured by strong gates to guard the entrance, was not open to the public, and any civilian wishing to go there was required to obtain permission (Anonymous 1844; Luffman 1789).

Sanitation was a particular problem at the dockyard at English Harbour because of its physically narrow boundaries, which allowed for diseases to spread easily among the crews on the ships. Due to the large number of naval personnel and the lack of tides at English Harbour, the dockyard was likely turned into a haven for disease and flies (Ross 1961). Visitors to English Harbour had labeled it the "most unhealthy place in the West Indies" (Ross 1961:97), and it was

commonly avoided whenever possible because of its reputation for making the men sick (Crewe 1993).

By 1814, Britain had gained the upper hand among the European colonial powers, and the economic importance of the Leeward Islands had diminished, resulting in the decline of the utility of the Royal Naval Dockyard at English Harbour (Richardson 1992). The dockyard served as a naval base, although in a diminished capacity, until 1889 when it was decommissioned (Weaver 2002).

2.2.3 The Inhabitants of Antigua and the Dockyard

In the mid-seventeenth century, Antigua's colonial population was small, with only 1,000–1,200 inhabitants. At this time, few enslaved labourers had been brought from Africa (Dunn 1972; Sheridan 1973). However, by 1678 the population of enslaved labourers of African descent was nearly equal to the population of European origin. The number of enslaved labourers on the island continued to increase to the point that one hundred years later, in the second half of the eighteenth century, the ratio of whites to blacks (i.e. those of African descent) reached 1 to 14 (Sheridan 1973). Male to female ratios were initially unbalanced among both black and white populations. By the end of the seventeenth century, 30% of the white population was female. Male enslaved labourers also outnumbered females during the same time period. By 1801, however, the number of black females was slightly more than the number of black males (Berleant-Schiller et al. 1995; Hart 1998; Higman 1984). Though the large majority of people of African descent in the late seventeenth century and early eighteenth century were enslaved, by the late eighteenth century there were some individuals categorized as “free coloured”. The number of “free coloured” people increased over time, some having been born into slavery and freed, and others born free (Berleant-Schiller et al. 1995; Hart 1998). Often, those who were freed or born free were individuals of mixed ancestry. These people were also more likely to live in urban areas and thus more likely to avoid the physically arduous demands of field labour (Richardson 1992). In 1805, 1,300 individuals were “free coloured,” a number that increased until emancipation in 1834 (Berleant-Schiller et al. 1995; Hart 1998), when Antigua became the first colony in the Caribbean to fully emancipate the enslaved population of the island (Nicholson 1986). At the time of emancipation, records showed that the enslaved population was comprised of 78.9% field labourers, 9.6% domestics, 7.7% tradesmen, 2.6% head people (or supervisors), and 1.2% dockworkers (Higman 1984).

As previously mentioned, there was a permanent naval garrison stationed at English Harbour, as well as military troops on the island in a variety of military installations (Buckley 1998; Ross 1961; Weaver 2002). During times of peace, however, the size of the garrisons in all of the West Indies tended to be quite small, often with one company of 35–100 men as the sole military presence on any particular island. Demands were made in the 1730s, after a slave revolt, that the number of men stationed at Antigua be no fewer than 400 (Buckley 1998). At English Harbour, men were employed as carpenters, smiths, boatswains, foremen, and shipwrights. Boys were also employed and living at the dockyard (Crewe 1993). Troops were dramatically increased during times of war, and the West Indies saw a great influx of military personnel after 1760 (Buckley 1998). At any time in the late eighteenth century there may have been between 2,000–3,000 men at English Harbour to man the ships at the dockyard, which could receive up to 10 men-of-war. In 1803 about 1,000 British troops were stationed at Shirley Heights, a military fort near the dockyard, in addition to the crews of the ships (Ross 1961). Thus, during the late eighteenth and early nineteenth centuries, there was a substantial European military presence on the island of Antigua to assist in the French Revolutionary and Napoleonic Wars (Buckley 1998; Ross 1961). Starting in the late eighteenth century, enslaved individuals of African descent also began to serve as soldiers in the West India Regiments. In 1800, the number of men in the West India Regiments was approximately 10,000. A company of these men was stationed at Antigua in the early nineteenth century (Dyde 1997).

Large numbers of naval and military personnel encouraged the presence of women at the dockyard area. These women were reputed to pass diseases on to the men staying at English Harbour through sexual contact, as well as provide them with rum in exchange for food, clothing, or bedding (Lloyd and Coulter 1961). In addition to the naval personnel living and working at the Dockyard, there was a presence of enslaved labourers. These individuals had not only been involved in the construction of the dockyard itself, but in the 1740s multiple acts were passed requisitioning more slave labour at English Harbour in preparation for war with France. Both male and female adult enslaved labourers worked at the dockyard from sunrise until noon, with a short break for breakfast between nine and ten o'clock, and then from two o'clock until sunset. For common labourers, the work at the dockyard was considered to be as difficult as work on the plantations. Enslaved labourers who did heavy manual work were well supervised, whereas those doing tradesmen's work were not as closely supervised (Gaspar 1985). Many of

the tradesmen were owned by the Navy, and called the King's Negroes (Crewe 1993; Nicholson 2002). In 1744, Charles Knowles, an officer of the Royal Navy purchased 12 enslaved labourers to be apprenticed to caulkers and carpenters at the dockyard in order to aid during a skilled labourer shortage (Crewe 1993). The King's Negroes were trained to carry out a variety of tasks at the dockyard, including masonry (Crewe 1993; Nicholson 2002). Both male and female enslaved labourers were owned by the Navy (Crewe 1993). These individuals may have been treated better than other enslaved labourers; although work was difficult, punishments seem to have been handed out infrequently (Nicholson 2002).

2.2.4 The Royal Naval Hospital and Cemetery

A hospital at English Harbour was initially built around 1745, at a time of considerable expansion of the naval dockyard, and was operated by civilian contract (Crewe 1993; Nicholson 1995). The care of the sick was entirely the responsibility of the individual contracted, who was to provide doctors and nurses, accommodation, medicine, food, and burial of the dead (Crewe 1993). The hospital was destroyed in 1772 by a hurricane. Until the hospital was rebuilt, a temporary hospital had to be used (Nicholson 1995). The temporary hospital was of poor quality and at the time English Harbour was considered a "grave of Englishmen" (Lloyd and Coulter 1961:135). Dr. Leonard Gillespie, surgeon and agent to the naval hospital at Fort-Royal on Martinique, even encouraged the closure of the hospital at English Harbour because of its horrific reputation, which was second in its infamy only to the hospital at Port Royal, Jamaica (Gillespie 1800). A hospital midden was discovered, and from it some artifacts salvaged, in 1980. The artifacts date to the time period of the new hospital, which was constructed in 1793 on the top of a hill, in the same location as the original hospital that had been destroyed. Figure 2.2 shows a map of the hospital buildings. Excavated artifacts demonstrate that patients of a variety of origins were treated at the hospital, including those from Britain, prisoners of French origin, and African-Caribbean members of the West India Regiments. Personnel of a variety of ranks were also treated there (Nicholson 1995). Findings at the associated cemetery, excavated from 1997–2001, suggest that the hospital treated all those who lived and worked at the dockyard, including children (Varney and Nicholson 2001). The hospital was expanded in the 1790s, and appears to have continued in operation until the 1820s (Nicholson 1995). In the nineteenth century it was described as "the iron hospital for the reception of invalid soldiers" (Anonymous 1844). Today, no structures remain of the hospital buildings (Nicholson 1995).

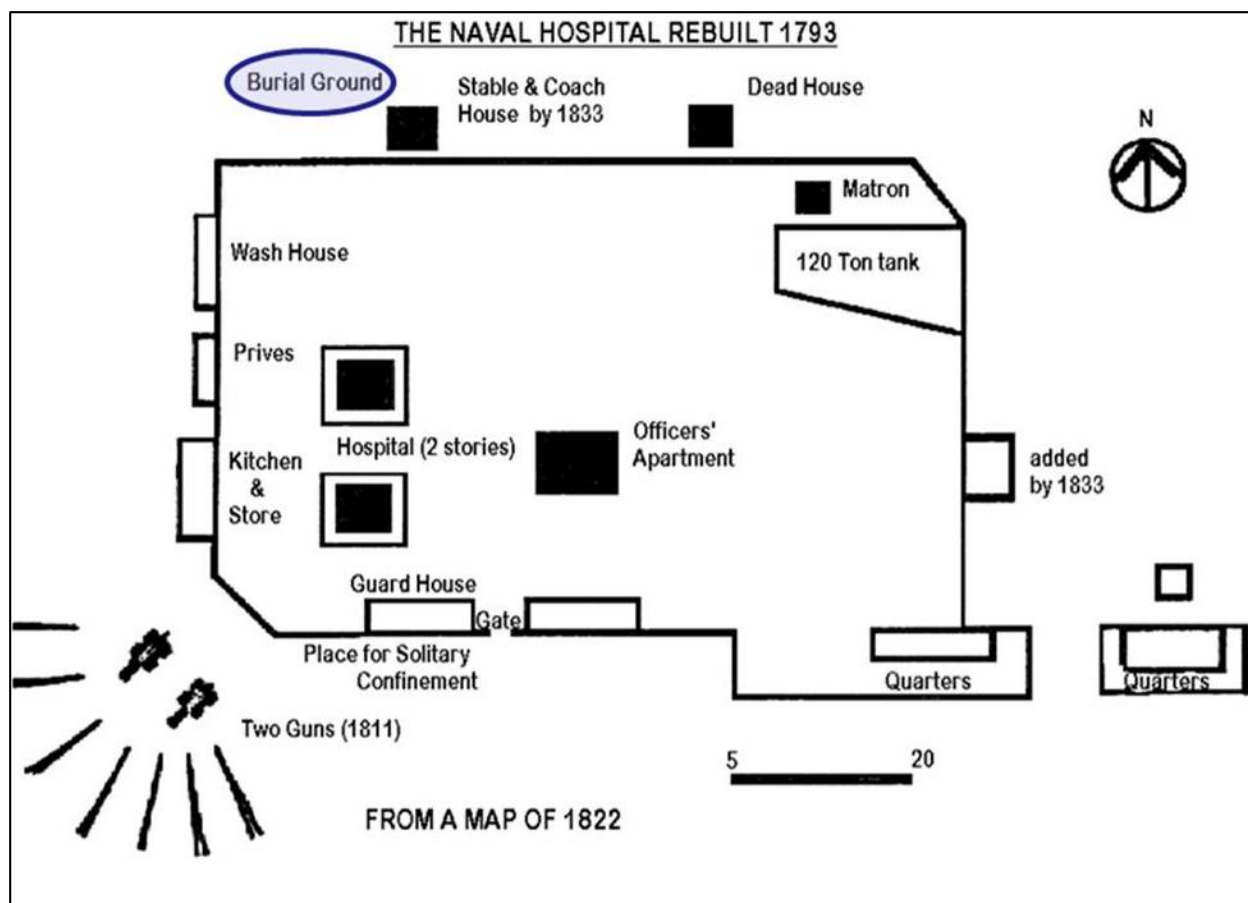


Figure 2.2 Map of the hospital with burial grounds indicated. Map from Nicholson (1995:8).

The hospital had an associated cemetery, in use from 1793–1822, for those individuals who died during their stay and were not afforded burial in a parish cemetery. These individuals were low-ranking officers, sailors, and labourers (Varney 2003). Due to modern urban development, much of the cemetery has been disturbed (Varney and Nicholson 2001). However, human remains were recovered from in situ contexts at the RNHC and the bone samples taken from these individuals were used to conduct this research. A more detailed description of archaeological investigations of the cemetery is presented in Chapter 4.

2.3 Diet, Health, and Lead Poisoning in the Colonial West Indies

2.3.1 Diet in the West Indies

Though the English colonists had to make many changes in their lifestyles when they migrated to the West Indies, one area in which they made a particular effort was to keep a diet similar to that which they had enjoyed in Europe (Dunn 1972). Maintaining a familiar diet was

not simply about the comforts of home in a strange land, but also part of the strict hierarchical social system created to keep the classes separate (Crabtree 1990; Dunn 1972; Twiss 2012). This allowed the plantation owners to eat foods that were different from the labourers who worked their land. Despite their desire to maintain their European diet, this was not always an easy task in the West Indies, given that many crops grown in England could not be cultivated in the Caribbean (Dunn 1972). Once land was turned over to growing sugarcane, it was necessary for most food to be imported from Britain, Ireland, and North America (Dirks 1978; Dunn 1972). At great financial cost, the colonists avoided tropical produce, with the exception of some tropical fruits, which were highly prized (Dunn 1972; Schaw 1922). Flour, bread, and beer were imported, despite the flour often being spoiled on arrival and the beer often spoiling quickly after arrival (Dunn 1972). Laws were put in place to force the colonies to import as many goods as possible from Britain, as a result of fears that local manufacturing would harm British industry. Several Navigation Acts were put in place beginning in the mid-seventeenth century to control trade by applying duties to imported foreign goods and preventing trade among other nations. Inhabitants of the West Indies, however, found ways to circumvent these regulations and access goods from North America in addition to what was permitted from Britain (Richardson 1992).

Though cattle and sheep were raised on the islands, the meat they provided was not of the best quality. Pork rivaled beef as a popular meat in the West Indies, though beef continued to be consumed despite the high price to obtain it fresh. Both poultry and pork in the West Indies were considered to be superior to that obtainable in England (Dunn 1972). The wealthier classes were able to provide a variety of different types of meats to guests (Dunn 1972; Schaw 1922). Salted meats and salt fish were imported, while local fish, though plentiful, was avoided by the wealthy colonists. Poor whites, in contrast, did not have the means to consistently maintain a European diet and often had to consume foods that the wealthy would not eat (Dunn 1972). When imported foods were not available on the island due to curtailed shipments, whites were able to purchase at the market vegetables and meats that had been grown and raised locally by enslaved labourers (Luffman 1789). Some foods were debatable as to their desirability, such as turtle, enjoyed by some but detested by others (Dunn 1972; Schaw 1922). Milk was most commonly provided by goats rather than cattle, cow's milk being reserved for the sick (Schaw 1922).

The colonists tended to be heavy drinkers, but again avoided many locally available options. Beer was a popular beverage, and once sugarcane cultivation was in place on the

islands, rum, a product of sugarcane, became an important liquor on the islands (Dunn 1972). Due to regulations stating that sugar could only be partially refined in the Caribbean, plantation owners were left with molasses, which they used to produce rum (Richardson 1992). Rum was often served as part of a punch that also included lime juice and sugar (Dunn 1972; Schaw 1922). It was especially popular among the lower classes due to its low price, while the wealthier colonists imported wine and liquor from Europe (Dunn 1972; Schaw 1922). Despite sugarcane being the principal crop grown in the West Indies in the eighteenth century, sugar was imported from England, where it had been refined for consumption (Richardson 1992; Schaw 1922).

Approximately 40,000 British military personnel were stationed in the Caribbean in the later years of the eighteenth century. Food had to be imported from Britain in order to feed the troops. Thus, large quantities of provisions were loaded onto ships carrying military personnel to the Caribbean not only to feed the men on board, but also to supply the garrison already living on the islands (Duffy 1987). Typical foods for the military in the West Indies at meal time were bread, peas, rice, and salted beef or pork, supplemented by vegetables purchased from the market (Buckley 1998). Although the men could purchase vegetables of their own accord, the military did not issue fresh vegetables or fruits as part of the rations (Dyde 1997). Dr. Gilbert Blane (1785:282), a Royal Naval physician, stated that the food seamen consumed was the most “unnatural” part of naval life. It was estimated that each man on board a ship required a ration of 3.5 lbs (1.6 kg) of pork, 14 oz. (0.4 kg) of beef, 7 lbs (3.2 kg) of flour, 6 oz. (0.2 kg) of butter, and 3 pts. (1.7 kg) of peas for one week (Duffy 1987), although officers and lower-ranking personnel did not always receive the same provisions. Onboard ships, officers sometimes had access to livestock, while seamen typically ate salted meats, which Blane considered to be particularly harmful to the men’s health if not used in moderation (Blane 1785). However, cattle and sheep were obtained by the Navy for its forces once at Antigua, when the Codrington estate on Barbuda could provide them (Dyde 2000). Other foods available to naval personnel were biscuits and bread. Bread was an important staple, though there were some issues with its preservation (Blane 1785). Locally available foods in the West Indies could be added to the regular rations of the military, including tomatoes, coconut, maize or maize bread, cassava, pumpkin, sweet potatoes, yams, and plantains (Buckley 1998). Molasses mixed with oatmeal, and sauerkraut served with rice were considered helpful in preventing scurvy. In fact the pickled cabbage became so important that it replaced a large quantity of oatmeal in the naval diet.

Different proportions of sauerkraut were served to men depending on whether it was a beef day, a pork day, or a peas day. Butter was advised against on expeditions to the tropics because it went rancid quickly. Butter in the West Indies was replaced by half a pound each of sugar and cocoa for each pound of butter (Blane 1785). Table 2.1 provides the daily allowance for each man in the Navy (Blane 1785:292).

Table 2.1 Daily Allowance of Provisions for Each Man in the Navy*

	Biscuit	Beer	Beef	Pork	Pease**	Oatmeal	Butter	Cheese
	lbs.	galls.	lbs.	lbs.	Pint.	Pint.	ozs.	ozs.
Sunday	1	1	-	1	half	-	-	-
Monday	1	1	-	-	-	1	2	4
Tuesday	1	1	2	-	-	-	-	-
Wednesday	1	1	-	-	half	1	2	4
Thursday	1	1	-	1	half	-	-	-
Friday	1	1	-	-	half	1	2	4
Saturday	1	1	2	-	-	-	-	-

*Table from Blane (1785: 292). ** Spelling of “peas” from original source.

Rum was particularly popular among soldiers and sailors, and became a standard drink consumed during times of peace and war alike (Richardson 1992). Due to the consumption of large quantities of salt from the salted meats and living in a hot climate, personnel were thirsty and often quenched this thirst with rum (Dyde 1997). Rum was introduced as part of the naval rations in the first half of the eighteenth century, and was initially served neat (Pack 1982). The daily ration was typically a half pint (284 mL); however, sometimes additional rum was smuggled aboard ships (Howard 2000; Pack 1982). To prevent drunkenness, it was ordered in the 1740s that rum must be mixed with water, along with sugar and limes when available, and provided in two servings during the day. The mixed beverage was called “grog,” and remained part of naval rations until 1970 (Pack 1982).

Sick naval personnel received “portable” soup and the preserved juice of oranges and lemons (Blane 1785; Lloyd and Coulter 1961). Other dietary suggestions made for the sick were wine, cocoa with sugar, pickled cabbage, potatoes, and regular salt provisions (Lloyd and Coulter 1961). Even though the sick received special provisions, they were still probably undernourished (Blane 1785). Blane (1785) noted that although this was of concern to several naval physicians, the only improvements made were to put raisins in the pudding for the sick, and to give them vinegar, which he considered an excellent preservative of health. The victualing contract with the hospital at English Harbour indicated that the sick were to receive fresh beef, mutton broth, bread, fresh meat, punch, butter, rice, eggs, and cheese (Crewe 1993).

There were two primary methods of provisioning enslaved populations in the West Indies. 1. On some islands, on land that could not be used for commercial agriculture, subsistence plots were allotted to enslaved labourers so that they could grow their own food, which allowed planters to supply a decreased quantity. 2. In cases where land was almost entirely used for sugarcane cultivation, the planter was responsible for feeding the enslaved and food had to be imported (Dirks 1978). The diet of the enslaved in the Caribbean was often greatly influenced by the weather affecting the region. Where enslaved labourers were permitted to maintain subsistence plots to produce some of their own food, bad crop years could be devastating. Because they were also highly dependent on imported foods from North America, poor weather in the West Indies often prevented imported food from arriving. Hurricane season, which prevented imports in the fall, was a difficult time for enslaved labourers and was a time of hunger (Dirks 1978; Richardson 1992). The diet of the enslaved was meager and consisted of maize, plantains, legumes, and sweet potatoes (Dunn 1972). They were also given imported salt fish of the lowest quality available in the quantity of a pound (0.45 kg) a week (Dirks 1978; Dunn 1972). Other imported food items included rice and horsebeans (Dirks 1978). An historical account from the late eighteenth century indicates that these foods were allotted to field labourers in the quantity of three to five quarts (3.4–5.7 L) of horsebeans, rice, or maize, with four salt herrings or two pounds (0.9 kg) of salt beef or pork per week. However, when yams, sorghum, sweet potatoes, plantains, or bananas were available, they could be used to substitute for the other standard staples (Luffman 1789). On occasion, diseased animals were also given to the enslaved for consumption when available, or the undesirable portions of a butchered animal were

passed on to the enslaved population (Dirks 1978; Dunn 1972). On special occasions, such as Christmas, an ox might be slaughtered and given to the enslaved labourers (Higman 1984).

Though Antigua had little land for cultivation of subsistence crops, on some plantations, enslaved labourers were able to grow maize, yams, potatoes, and sorghum (Sheridan 1957). In other cases, overseers in Antigua allowed enslaved labourers to raise small livestock, such as pigs, goats, chickens, and other fowl (Dirks 1978). Often, the produce grown by the enslaved in Antigua was sold at the Sunday market in St John's (Luffman 1789). Rations of rum and molasses were also provided (Dunn 1972). It has been suggested that neither the caloric intake nor the protein content of enslaved labourer diet were sufficient for the physical labour being carried out (Dirks 1978). The Leeward Islands slave law of 1798, outlining the weekly provisions allotted to the enslaved, was generally consistent with the diet described above for all of the West Indies (Higman 1984).

Plantation enslaved labourers who worked the fields fared much worse, in terms of diet, than those who worked as artisans, fishermen, or as other types of skilled labourers (Richardson 1992). Tradesmen and head people were often given more provisions than field labourers even though they did less physical labour. The types of foods provided to, or purchased by, enslaved labourers, whether working on the plantation or in an urban setting, were likely very similar to those eaten by field labourers, including sorghum, yams, or maize as basic staples (Higman 1984). There is little documentation regarding the diet of enslaved labourers owned by the military (Varney 2003). It is likely, however, that enslaved labourers belonging to the military consumed similar foods to military personnel, but in smaller quantities (Buckley 1998). Buckley (1998) suggested that enslaved labourers owned by the military received three-quarters the ration that a British soldier would receive. Blacks serving in the West India Regiments, on the other hand, though enslaved, received the same rations as white soldiers, and they often sold their rations to obtain fresh vegetables and other foodstuffs (Dyde 1997). Historical documentation suggests that the King's Negroes also received rum and grog (Nicholson 2002).

2.3.2 Health and Lead Exposure in the West Indies

The colonists suffered many of the same health problems in the West Indies as the military personnel and the enslaved labourers. Diseases endemic to the region included malaria, yellow fever, yaws, parasites, dropsy (water retention), and dysentery. The health problems they experienced were also heightened by excessive drinking and attempts to retain unsuitable

cultural practices, such as diet, dress, and housing, in a climate that was unlike that in Britain. Some colonists also suffered from sexually transmitted diseases, which were then spread to enslaved labourers (Dunn 1972).

A legitimate fear of being sent to the West Indies permeated the ranks of the British military, as it was considered to be akin to a death sentence (Buckley 1978, 1998; Gillespie 1800). The region had justly earned the epithet “the grave of the British army” (Buckley 1978:326). This was because of the frequency with which disease crippled the troops stationed in the Caribbean, the number of cases far outweighing casualties produced as a result of battle (Bell 1791; Buckley 1978; Duffy 1987; Dyde 1997). The losses occasioned between 1793 and 1815, during the French Revolutionary and Napoleonic Wars, were at the time the worst the British forces had ever experienced. Less than 10% of the military casualties in the last decade of the eighteenth century, which numbered 75,000 deaths, was the result of armed conflict (Buckley 1978). It is likely that even before departing from Britain, military recruits were not in perfect health, and their condition only worsened during the long journey overseas (Buckley 1978; Duffy 1987). Typhus, fever, and scurvy were major ailments for seamen and often resulted in death. Once in the West Indies, the hot climate and poor sanitary conditions led to many cases of dysentery (Duffy 1987). However, the islands of the Caribbean held even more devastating illnesses for the troops, who were exposed to malaria and yellow fever there (Buckley 1978; Duffy 1987; Lloyd and Coulter 1961). Both diseases, transmitted by mosquitoes, had the capacity to kill, and military personnel were especially at risk because barracks were often built in close proximity to swamps where mosquitoes bred (Duffy 1987; Dunn 1972). Poor nutrition may have played a significant role in the susceptibility of the sailors to infectious diseases, given the link between nutritional deficiencies and vulnerability to illness (Guerrant et al. 2000; Solomons 2000). A variety of other health conditions, including heat-stroke, left the troops in poor condition and exposed to more serious ailments (Duffy 1987).

Other major health concerns for the British military, due to the ready availability of rum in the West Indies, were chronic alcohol poisoning and lead poisoning (Buckley 1978). As previously noted, in the 1740s, the Navy issued orders that rum should be watered down, as the commanders were aware that excessive alcohol consumption had a negative impact on the men’s health (Crewe 1993; Pack 1982). Dyde (1997:17) went so far as to state that there was a “widespread addiction to a substance known as ‘new rum’”, the first distillate of rum production.

Seamen had a reputation for drinking heavily, and in the late eighteenth century, physicians had been made aware that drinking rum was especially bad for one's health because it was exposed to lead during the distillation process (Blane 1785; Buckley 1978; Lloyd and Coulter 1961). Drinking was also considered problematic because it made the men more prone to diseases and accidents that could make them invalids (Duffy 1987). Figures are not available to attest to the number of troops who could not perform their duties as a result of alcohol and lead poisoning; however, it has been suggested that these numbers might outweigh the number of men made invalid by malignant fevers (Buckley 1978). Although not necessarily attributed to lead poisoning at the time of diagnosis, descriptions of symptoms suggested that between 1793 and 1815, several thousand men in the military were discharged for conditions that appear to be related to lead exposure (Buckley 1978). Dry bellyache, or the West India dry gripes, described as causing discomfort of the bowels, anxiety, and restlessness in its early stages, had the potential to become a more severe health concern, and could cause vomiting, severe pain of the bowels, convulsions, paralysis of the hands, feet, and arms, and sometimes paralysis of the entire body (Cadwalader 1745; Hunter 1796). In the most severe cases, the illness could prove fatal (Hunter 1796). During the first half of the eighteenth century, the cause of these symptoms was not certain, though Cadwalader (1745:4) suggested the cause to be "an obstructed perspiration, by being too much exposed to a moist night air, and cold raw winds; hard drinking, especially drams or strong punch..." Though the condition had not been related specifically to lead at the time, the connection to rum had been proposed. By the late eighteenth century, however, it was clear to some physicians that lead, in all forms and in whatever manner introduced to the body, was toxic and was indeed the cause of the symptoms of the dry bellyache (Hunter 1796). In the West Indies and Jamaica it was considered that new rum was the primary source of lead poisoning (Blane 1799; Howard 2000; Hunter 1796). Hunter (1796:210) stated that "the new rum, distilled in improper vessels, appears to be the vehicle in which it finds admission. I have not yet met any other facts or observations, to induce me to change the opinion I have advanced on this subject." Despite Hunter's conclusions, and Blane's support for them, not all physicians at the time believed that lead was responsible for the dry bellyache and lead-contaminated rum continued to be available to the military (Buckley 1978). Although the naval population on Antigua was probably exposed to lead primarily through contaminated rum, lead was also used in everyday

items such as cooking and eating utensils, ceramics, and water catchments (Eisinger 1982; Handler et al. 1986).

Other white inhabitants of the islands also suffered lead poisoning by consuming large quantities of rum. Although wealthier colonists tended to consider rum a drink of the lower classes and preferred other alcoholic drinks (Dunn 1972; Schaw 1922), dry bellyache was not unheard of among the civilian population of the West Indies (Dunn 1972). In fact, Towne (1726:87) wrote of the frequency with which people in the West Indies suffered from the dry bellyache: “This is so popular a disease in the Leeward Islands that it may very justly be reckoned as endemic in them, most people there at one time or other having felt its cruelty.”

The health of enslaved labourers tended to be poor, as planters and overseers had calculated that the cheapest labour option was to push the enslaved to the utmost so that they did not have the opportunity to grow old and lose the ability to work. Enslaved labourers working on sugar plantations thus tended to have very short life expectancies during the eighteenth century (Dirks 1978; Hart 1998; Newton 1788; Richardson 1992). In other cases, however, some plantation owners expressed concern that their enslaved labourers be well-fed and cared for (Gaspar 1985). Overworking was one major contributor to poor health, but malnutrition and starvation were also substantial concerns among the enslaved populations. These individuals were of least importance during hard times and, when it was difficult to obtain sufficient quantities of provisions for the islands, it was not unheard of for the enslaved to starve to death (Dirks 1978; Richardson 1992). Malnutrition, as previously noted, increased susceptibility to disease, and also encouraged low birth rates (Dirks 1978; Sheridan 1985). Health conditions common among field labourers included skin ulcers and lesions, “sugar disease” (beriberi-pellagra syndrome) brought on by consumption of high quantities of sugarcane juice, and illness of the bowels. In particular, diarrhea, caused by a variety of health problems, was a major cause of death of the enslaved in the West Indies. In 1779, on Antigua alone, 4,500 enslaved labourers died when a wave of bowel illness afflicted the enslaved population, likely related to a drought that same year which resulted in severely restricted rationing of food (Anonymous 1844; Dirks 1978). Other health conditions that affected the enslaved population were yellow fever, smallpox, influenza, yaws, and other infectious diseases (Dirks 1978). Little has been documented on the health of the King’s Negroes, except that, in general, the health of the dockyard labourers, both black and white, tended to be poor and was of concern for those in

charge as it reduced the work rate (Crewe 1993). Despite the poor health of much of the enslaved population in the West Indies, blacks were often seen as healthier and more robust than white British troops when serving as soldiers. For this reason, regiments of black soldiers were formed in the West Indies in the late eighteenth century (Dyde 1997).

Though less historical documentation exists to attest to the fact, due to the marginalization of the enslaved labourer population and less careful attention paid to their health, lead poisoning was undoubtedly also quite problematic for these individuals. Rum would have been an important source of lead exposure to the enslaved labourers given that it often formed part of their rations (Dirks 1978; Higman 1984). However, those individuals who worked in the processing of sugarcane into raw sugar products would likely have been occupationally exposed to lead (Handler et al. 1986). Both rum distillation equipment and sugar processing equipment were manufactured with some quantity of lead during the colonial period, thus exposing people to this harmful toxin (Eisinger 1982; Handler et al. 1986; Sheridan 1973; Wedeen 1984). It was the enslaved labourers on plantations that processed sugarcane, and therefore they would have been exposed to lead vapours in the sugar boiling houses (Sheridan 1973). On some plantations, enslaved labourers were also given the task of distilling the rum. Rum mixed with water and molasses was given to enslaved labourers on many plantations due to its high-caloric content (Dirks 1978; Higman 1984).

2.4 Summary

The island of Antigua during the colonial period was both the permanent and temporary home to a variety of different social groups including, but not limited to, white colonists, white military and naval personnel, black and mixed ancestry enslaved labourers, as well as black and mixed ancestry freedmen. Until the second decade of the eighteenth century, the West Indies was nearly perpetually in a state of turmoil, with the presence of war or the threat of war, slave uprisings, climatic disasters, and endemic diseases. It was a place few people wished to go, freely or by force.

An important military installment on Antigua was the Royal Naval Dockyard, which, thanks to its geographic advantages, allowed a year-round presence of British ships in the West Indies to protect British possessions from threat. Included in the infrastructure of the dockyard was the Royal Naval Hospital and its cemetery. The hospital had a reputation of being of particularly poor quality, and death rates on Antigua were high.

The diet of the inhabitants of Antigua was quite varied through both imported foods and locally procured provisions. Primarily it was only the wealthy whites who could afford to be choosy about the foods they consumed, preferring to eat a diet similar to that which they had eaten in Britain. Lower-ranking military personnel ate rations provided for them by the government, primarily imported foods, but were able to supplement this diet with foods purchased locally. Enslaved labourers ate a combination of imported foods provided by their owners, but also had access to foods which they had grown or raised themselves, or which they had procured from the market.

Health in the West Indies tended to be poor among all social groups. White colonists suffered from tropical diseases, and had a tendency to consume too much alcohol. The military was afflicted with similar health concerns from endemic diseases, as well as lead poisoning due to the consumption of lead-contaminated rum. Enslaved labourers suffered from malnutrition, endemic diseases, and also from lead-related health conditions. In general, the West Indies during the colonial period was a place rife with disease and illness.

This chapter presented historical documentation of diet and lead exposure for individuals living in the West Indies during the colonial period. However, in archaeological contexts, diet must be reconstructed using a proxy, in this case stable isotopes. Long-term lead accumulation may also be quantified from skeletal remains via trace element analysis. The principles behind these methods of analyzing skeletal material are presented in Chapter 3.

Chapter 3: Stable Isotope Analysis, Trace Element Analysis, and Lead in Bone

3.1 Introduction

This chapter presents a brief overview of the principles of stable isotope analysis, trace element analysis, specifically as it pertains to studies of lead (Pb), and the incorporation of lead in bone. The overview of stable isotope analysis covers the distinction between C₃, C₄, and CAM plants, as well as the use of stable carbon and nitrogen isotopes for dietary reconstruction of past populations. Stable isotope studies most relevant to the research conducted on the individuals interred at the Royal Naval Hospital Cemetery (RNHC) are presented. This chapter also focuses on the principles of trace element analysis and the challenges faced in its application to archaeological problems. In particular, the study of lead from archaeological bone is reviewed. Finally, a summary of the incorporation of lead into bone and the health effects of lead is presented. The objective of this chapter is to provide the necessary background for the methodologies covered in Chapter 4, as well as summarize studies relevant to the research presented in this thesis.

3.2 Principles of Stable Isotope Analysis and Paleodietary Reconstruction

3.2.1 Introduction to Stable Isotope Analysis

Stable isotope analysis has become an essential and dependable tool in paleodietary reconstruction from human and animal tissues (Katzenberg 2008). Since the late 1970s, analysis of stable isotopes has been applied to archaeological remains in order to reconstruct diet as a primary area of interest (van der Merwe and Vogel 1978; Vogel and van der Merwe 1977). Dietary reconstruction via stable isotope analysis has then also been applied to further elucidate areas of past lifeways such as infant weaning (Fogel et al. 1997; Herring et al. 1998), migration and residence studies (Cox and Sealy 1997; White et al. 1998), and gender and class differences (Ambrose et al. 2003; Le Hurray and Schutkowski 2005; White et al. 2004). The study of stable carbon (C) and nitrogen (N) isotopes has provided researchers with the capacity to determine, in a general sense, what past people ate and, with advancing techniques, changes in diet over time through the study of both organic and inorganic components (i.e. collagen and apatite

respectively) of skeletal tissues (Ambrose 1993; Katzenberg 2008). Through careful study of stable isotopes in bones and teeth, archaeologists are able to investigate the multiplicity of ways that knowledge of the foods people ate in the past can lead to a more thorough understanding of how people interacted with their environment at different stages during their lifetimes. This section presents a brief review of the principles of stable isotope analysis of human bone tissues, as well as an overview of past studies that have carried out paleodietary reconstruction. Finally, investigations of stable isotopes in geographical areas and time periods of particular relevance to the research presented in this thesis are examined. Studies of relevance include dietary reconstructions in Europe, Africa, the Caribbean, and North America during the seventeenth, eighteenth, and nineteenth centuries. These studies are of particular consequence since the RNHC, located in the West Indies and dating to the colonial period, contained the remains of individuals of both European and African ancestries.

3.2.2 Stable Isotope Theory

Paleodietary reconstruction is based on the knowledge that there is a systematic difference, due to isotopic enrichment or depletion, between the tissues of the consumer and its diet (DeNiro and Epstein 1978). Elements are determined based on the number of protons in their nuclei, but they may have several isotopes. These isotopes are variations of elements based on the number of neutrons in their nuclei, which result in a different mass. The isotopic mass is indicated as a superscript beside the symbol for the element. There are three isotopes of carbon: ^{14}C , which decays over time, and is therefore “unstable” or radioactive, and ^{12}C and ^{13}C , both of which maintain a constant ratio of protons and neutrons over time, and are thus stable (Hoefs 2009).

Due to differences in atomic mass among isotopes, they have different physical and chemical properties. Lighter isotopes react faster during a chemical reaction than heavier isotopes (Hoefs 2009). During plant photosynthesis, carbon fractionation occurs. Fractionation is the result of the different reaction rates of isotopes due to atomic mass, and is the primary source of variation in isotope ratios. Thus, during photosynthesis the $^{13}\text{C}/^{12}\text{C}$ ratios are lowered in comparison to atmospheric CO_2 (Smith and Epstein 1971). Plants discriminate differently against ^{13}C during photosynthesis, which facilitates their classification as C_3 (Calvin-Benson), C_4 (Hatch-Slack), or CAM (crassulacean acid metabolism) plants. Isotopic composition of plants is typically expressed as a $\delta^{13}\text{C}$ value in per mil (‰). This is reflective of the $^{13}\text{C}/^{12}\text{C}$ ratio of the

sample compared to the standard, which is most commonly belemnite from the Pee Dee formation in South Carolina (PDB) (O'Leary 1981). PDB was exhausted several decades ago, and although new standards are available, PDB remains the international standard to which δ -values are compared (Hoefs 2009). In comparison to the PDB standard, organic matter is depleted in ^{13}C , thus the values for $\delta^{13}\text{C}$ are negative (O'Leary 1981).

Most plants use the C_3 photosynthetic pathway, which results in less positive $\delta^{13}\text{C}$ values, with an average $\delta^{13}\text{C}$ value of -26.5‰. C_4 plants, in contrast, have more positive $\delta^{13}\text{C}$ values, and include grasses adapted to dry environments, such as millet and maize. The average $\delta^{13}\text{C}$ value for C_4 plants is -12.5‰. $\delta^{13}\text{C}$ values for C_3 and C_4 plants do not overlap. CAM plants, which include succulents and desert plants, are intermediate to C_3 and C_4 plants in terms of $\delta^{13}\text{C}$ values (O'Leary 1988; Smith and Epstein 1971). Because the range of values for C_3 plants and C_4 plants does not overlap, this information can be used to reconstruct human diet from preserved tissues based on proportions of these types of plants consumed in life. In general, researchers have found that $\delta^{13}\text{C}$ values of bone collagen are approximately 5‰ greater than those of their diet (Katzenberg 2008). $\delta^{13}\text{C}$ values not only reflect consumption of terrestrial plants, but can also aid in the distinction between marine and terrestrial diets. Animals feeding exclusively on marine foods have less negative $\delta^{13}\text{C}$ values than animals feeding exclusively on terrestrial foods; however, there is an overlap in these values of approximately 4.5‰ (Schoeninger and DeNiro 1984). The difference in $\delta^{13}\text{C}$ values between marine life and terrestrial life reflects an approximately 7‰ difference between seawater bicarbonate and CO_2 in the atmosphere (Chisolm et al. 1982). $\delta^{13}\text{C}$ values for marine diets are similar to those for terrestrial diets that include C_4 plants. Thus, if both marine and C_4 foods are available to a population, $\delta^{13}\text{C}$ values cannot be used as the sole indicator of diet, and must be combined with stable isotope values from nitrogen (Schoeninger and DeNiro 1984).

Stable nitrogen isotopes can be used to determine trophic level (DeNiro and Epstein 1981) and to distinguish between marine and terrestrial diets (Schoeninger and DeNiro 1984). There are two isotopes of nitrogen in the atmosphere: ^{14}N and ^{15}N (Hoefs 2009). The standard against which $\delta^{15}\text{N}$ values are measured is atmospheric nitrogen (N_2) which is 0‰. Since plants and animals tend to be enriched in $\delta^{15}\text{N}$ compared to the atmosphere, values are positive (DeNiro and Epstein 1981). Leguminous plants have lower $\delta^{15}\text{N}$ values because they take up nitrogen from the atmosphere with the aid of symbiotic bacteria living in the roots, while non-leguminous

plants have higher $\delta^{15}\text{N}$ values because they take up nitrogen from the soil in the form of ammonium and nitrates (Delwiche and Steyn 1970; DeNiro and Epstein 1981; Santi et al. 2013). Leguminous plants, prior to the use of chemical fertilizers (which reduce the difference in $\delta^{15}\text{N}$ values between leguminous and non-leguminous plants), typically had $\delta^{15}\text{N}$ values that ranged from -3.0‰ to 5.0‰ (average of 1.0‰), while non-leguminous plants ranged from -4.0‰ to 10.0‰ (average of 9.0‰) (DeNiro 1987). As trophic levels increase, from plant to herbivore to carnivore, $\delta^{15}\text{N}$ values increase by approximately 3‰ above the values for their diet (DeNiro and Epstein 1981). Thus, an herbivore consuming leguminous plants would have lower $\delta^{15}\text{N}$ than an herbivore consuming non-leguminous plants, while a carnivore would have even higher $\delta^{15}\text{N}$ values than the herbivores (Katzenberg 2008). Schoeninger and DeNiro (1984) demonstrated that there is a 4‰ increase in mean $\delta^{15}\text{N}$ values in marine plants over terrestrial plants, and a 9‰ increase in mean $\delta^{15}\text{N}$ values for marine animals over terrestrial animals. The small difference in $\delta^{15}\text{N}$ values between plants in the two environments, due to marine plants taking up dissolved inorganic nitrogen rather than nitrogen directly from the air or soil (Peters et al. 1978), increases at higher trophic levels likely as a result of selective feeding by animals and because there is at least one additional trophic level in the marine system compared with the terrestrial system (Schoeninger and DeNiro 1984). Thus, humans who consume more terrestrial meat will, in general, have higher $\delta^{15}\text{N}$ values than those who consume less meat, and humans who consume marine resources will have even higher $\delta^{15}\text{N}$ values. Ambrose (1991) suggested, however, that caution must be used with foodwebs from different ecosystems because of the differences in nitrogen stepwise enrichment between arid and hot climates vs. cool and moist climates. This is discussed further in section 3.2.6.

3.2.3 Early Studies of Stable Isotopes in Archaeology

Stable isotope analysis emerged as an area of investigation in the field of archaeology in the mid-1970s, with a variety of studies aiming to reconstruct diet of past humans and animals (van der Merwe and Vogel 1978; Vogel and van der Merwe 1977). Vogel and van der Merwe (1977) studied bone samples from hunter-gatherers and horticulturalists in New York State to determine the effects the introduction of maize, a C_4 plant, had on the isotopic composition of bone collagen. They found that the hunter-gatherers did not consume C_4 plants, as expected, while the horticulturalists had much more positive $\delta^{13}\text{C}$ values, which the authors attributed to the increased consumption of maize. So long as sufficient information was available on edible

plants in a geographic area, the authors considered that isotopic analysis could provide a general indication of what type of plants were being consumed. Further study of human bone collagen by van der Merwe and Vogel (1978) in Ohio and Illinois also demonstrated a difference in isotopic composition of bone between hunter-gatherers who relied primarily on C₃ plants and horticulturalists who consumed large quantities of maize. These early studies focused entirely on stable carbon isotopes from bone collagen. Future studies would additionally examine stable nitrogen isotopes (Commendador et al. 2013; Eriksson and Liden 2013) and carbon isotopes from bone apatite (Harrison and Katzenberg 2003; Hedman et al. 2002) to achieve more accurate interpretations of isotopic data.

Controlled feeding experiments were another area of great importance in early studies of stable isotope analysis in order to apply the knowledge obtained to archaeological contexts. These experiments were conducted to determine the effect diet had on the isotopic composition of animal tissues for both carbon (DeNiro and Epstein 1978) and nitrogen (DeNiro and Epstein 1981). Animals, including shrimp, various types of insects, and mice were fed controlled diets appropriate to the species in question, including algae, lettuce, meat, and grains. In both cases the authors confirmed that carbon and nitrogen stable isotope analysis could be used for the investigation of diet in fossil specimens (DeNiro and Epstein 1978, 1981).

Further studies strove to differentiate consumption of marine vs. terrestrial foods through analysis of carbon isotopes (Chisholm et al. 1982; Schoeninger and DeNiro 1984; Tauber 1981). Tauber (1981) found that, although there could be some complications in interpretation when both C₄ plants and marine foods were consumed, C₄ plants were not eaten in prehistoric northwestern Europe, and therefore, elevated $\delta^{13}\text{C}$ values in archaeological human bone could be attributed to marine resources. This paleodietary reconstruction allowed for a comparison of Mesolithic to Neolithic inhabitants of Denmark. Chisholm et al. (1982) were in accord with Tauber that in the absence of C₄ plants, as was the case in prehistoric British Columbia, elevated $\delta^{13}\text{C}$ levels indicated consumption of marine protein.

Stable nitrogen isotopes and their relationship to trophic level were another area of research in the 1980s. As previously discussed, Schoeninger and DeNiro (1984) demonstrated a difference of 9‰ between mean $\delta^{15}\text{N}$ values of marine and terrestrial animals, and a 4‰ difference between mean $\delta^{15}\text{N}$ values of marine and terrestrial plants, with marine life forms having, in general, the higher $\delta^{15}\text{N}$ values. In contrast to this general trend, fish from reef

ecosystems had much lower $\delta^{15}\text{N}$ values than other fish due to the presence of blue-green algae, which fix large quantities of nitrogen that are depleted in $\delta^{15}\text{N}$, with these low $\delta^{15}\text{N}$ values being passed up the reef food chain (Schoeninger and DeNiro 1984; Stewart 1978). Again this demonstrated to researchers the necessity of understanding the available resources that people may have consumed in order to interpret the stable isotope signatures.

3.2.4 Tissues Used in Archaeological Stable Isotope Analysis

Bone is composed of an organic component and an inorganic mineral component, comprising 30% and 70% of dry bone weight respectively. The organic component is primarily made up of collagen, a protein, while the mineral component is primarily made up of hydroxyapatite, a mineral principally consisting of calcium and phosphate (Triffitt 1980). Carbon and nitrogen isotope analysis may be carried out on bone collagen (Katzenberg 2008). However, collagen degrades over time as a result of the activities of microorganisms in the burial environment, which can affect the stable isotope signatures from the bone (Child 1995). Carbon isotopes from the mineral component of bone are also used in dietary investigations. Stable isotope analysis from the organic and mineral components of bone provide different dietary information. In the mineral component of bone, carbonate (CO_3), is a source of carbon that can be used for isotopic analysis (Ambrose and Norr 1993). Some debate surrounded the use of bone apatite for analysis because of potential diagenetic, or postmortem geochemical changes to the mineral component of bone, resulting from interactions between bone and the burial environment (Radosevich 1993; Schoeninger and DeNiro 1982). However, laboratory experiments have provided methods for proper cleaning of the bone in order to remove soluble carbonates to reduce these types of issues (Krueger 1991). Methods to address the effects of diagenesis have been demonstrated to remove contaminants from bone that has been well preserved, but are not sufficient to fully clean poorly preserved bone (Nielsen-Marsh and Hedges 2000). Researchers have also demonstrated that the carbon found in collagen primarily reflects the protein component of an individual's diet, whereas the carbon found in apatite reflects the diet as a whole, including the carbohydrate, lipid, and protein portions of the diet (Ambrose and Norr 1993). Organic and mineral components of both bones and teeth have been utilized in stable isotope analysis (Katzenberg 2008).

3.2.5 Sample Preparation for Isotope Analysis

In order to conduct stable isotope analysis on bone, it is necessary to separate the desired component, collagen or carbonate, from the rest of the tissue. Katzenberg (2008) reviewed several methods of collagen separation, noting that different methods are necessary when bone preservation is poor. The proposed methods aim to remove the mineral component, typically through acid dissolution, as well as any organic matter that may have been deposited in the burial environment. This is done by placing small pieces of bone in one molar hydrochloric acid to remove bone mineral, and then placing the bone in sodium hydroxide to remove organic contaminants. Then the collagen is freeze-dried. Ambrose (1993) suggested that compact lamellar bone rather than cancellous bone is the most appropriate type of bone to use because it is less vulnerable to contamination. The preservation of collagen is judged based on the C:N ratio, which should fall between 2.9 to 3.6, given that the C:N ratio in bone that has never been buried is 3.2. However, C:N ratios that fall above 3.4 may reflect lipid, carbonate, or humic acid contamination of the collagen (DeNiro 1985). Multiple methods of determining the integrity of collagen should be used and include, but are not limited to, identifying the concentrations of collagen, carbon, and nitrogen (Ambrose 1990; DeNiro and Weiner 1988; van Klinken 1999). It is essential to remove contaminants properly because they may affect $\delta^{13}\text{C}$ values.

Isolation of carbonate from bone mineral is conducted by removing organic material and any carbonate that may have been deposited postmortem. Carbonate is separated by grinding bone and soaking it in sodium hypochlorite, which removes the organic component of bone. One method of removing carbonate resulting from postmortem contamination is to soak the sample in 1.0 M acetic acid for 24-36 hours (Lee-Thorp and van der Merwe 1991). Some researchers suggested, however, that a 1.0 M acid is too harsh and leads to loss of much of the sample. Thus, they proposed the use of a 0.1 M acetic acid, especially for recent bone, and a treatment time of no longer than 4 hours (Garvie-Lok et al. 2004). Carbonate from the burial environment is typically from the carbonate in soil and groundwater which can be deposited in voids in the mineral structure of bone. This carbonate contamination results in more negative $\delta^{13}\text{C}$ values (Ambrose 1993).

3.2.6 Paleodietary Studies from Carbon and Nitrogen Stable Isotope Analysis

Paleodietary studies have investigated a variety of different dietary regimes in order to determine the use of C_3 vs. C_4 plants, marine vs. terrestrial diets, and trophic level along the food

chain. A popular area of study, particularly in North America, was the determination of maize content and time of introduction of maize into the diets of past groups, since maize in the diet could be distinguished from diets consisting primarily of C₃ plants. As previously noted, van der Merwe and Vogel (1978; Vogel and van der Merwe 1977) were the first to demonstrate this principle in past North American populations. Studies that sought to determine maize usage through analysis of stable carbon isotopes allowed researchers to distinguish between diets that contained no maize at all (Bender et al. 1981) and consumption of increasing quantities of maize over time as it became a common dietary staple among North American populations (Schwarcz et al. 1985). The addition of $\delta^{13}\text{C}$ analysis from bone apatite permitted investigators to establish the consumption of small quantities of maize that were not discernible from the $\delta^{13}\text{C}$ values of bone collagen alone. The latter line of inquiry is based on the fact that apatite is more sensitive to small changes in the carbohydrate component of diet than is collagen (Harrison and Katzenberg 2003). Investigations in the area of maize introduction via stable isotope analysis resulted in findings that maize adoption and usage were quite variable among different groups and regions, reinforcing the notion that a variety of factors, including social, economic, and environmental, had a considerable influence on changes in dietary regimes, and though groups may have consumed similar foods, variation was frequent and expected (Schoeninger 2009).

Many of these studies of maize were conducted on populations that consumed no other C₄ plants as part of their diet. Application of stable isotope analysis to other regions, such as the American Southwest, where C₄ plants in addition to maize, as well as CAM plants were consumed, presented a different set of challenges for researchers (Katzenberg 2008). Matson and Chisholm (1991) discussed the potential problems that arose in their investigation of maize agriculture among the Anasazi as a result of the population having access to C₄ plants in addition to maize, as well as animals that had fed on C₄ plants. In this case, the researchers determined that these additional sources of C₄ plants in the diet would not have a substantial effect on the interpretation of maize consumption for the region if maize was consumed in large quantities while the other C₄ plants were limited in quantity. However, it became clear that the diet of the animals consumed by humans could have an impact on the stable isotope signatures if the animals were also consuming C₄ plants, because this would result in more positive $\delta^{13}\text{C}$ values in their tissues that would be passed on to the tissues of humans that consumed them.

Differentiating between consumption of C₃ and C₄ plants has been a major focus of paleodietary

studies, but these distinctions are not the only kinds of information that can be obtained from stable isotope analysis. More complicated dietary patterns often require the use of stable carbon isotopes from both bone collagen and apatite, and stable nitrogen isotopes to develop a more complete understanding of the diets of past populations.

Research into the consumption of C₃ and C₄ plants relies heavily on ratios of ¹³C/¹²C. Nitrogen isotopes, in contrast, offer the opportunity to distinguish between trophic levels based on a stepwise enrichment of $\delta^{15}\text{N}$ as one moves up the food chain. Studies to determine trophic levels are ideally carried out by analysis of a variety of plants and animals from an ecosystem in order to compare them to nitrogen values in remains of humans who lived in the same environment (Katzenberg 2008). Although the concept behind using $\delta^{15}\text{N}$ to determine trophic level in archaeological contexts appears to be straightforward, there are a variety of factors that can have a substantial effect on the stable isotope signatures. A great deal of variation in $\delta^{15}\text{N}$ values occurs between different types of ecosystems and even within an ecosystem as a result of the physiology of different animals. These variations are often reflected by differences between wet and arid regions, but also can be seen between herbivores that are drought tolerant and those that are not. All of these factors have an effect on $\delta^{15}\text{N}$ values in human remains (Ambrose 1991). Ambrose (1986) found that differences in $\delta^{15}\text{N}$ values for different populations in Africa may have been as a result of climate and water stress rather than dietary differences. Sealy et al. (1987) also examined the challenges presented when studying populations in arid ecosystems. They found that, although trophic level patterning for marine organisms was consistent with previous studies, the patterning for terrestrial organisms depended on the amount of rainfall in the area, with $\delta^{15}\text{N}$ values increasing in herbivores living in low rainfall regions, potentially because of retention of the heavier nitrogen isotope in urea, a chemical compound found in urine and through which nitrogen is excreted from the body. Nitrogen conservation is necessary in areas of low rainfall because the diet of herbivores tends to have poorer quality protein than that in watered areas. Schwarcz et al. (1999) suggested that high $\delta^{15}\text{N}$ values were both as a result of ¹⁵N depleted urea in herbivores and high $\delta^{15}\text{N}$ values in plants growing in arid regions. Thus, in environments where animals may have suffered water stress and as a result have high $\delta^{15}\text{N}$ values, stable nitrogen isotope analysis should not be used to differentiate between marine and terrestrial resources in the diet because of overlapping $\delta^{15}\text{N}$ values. They could, however, be

used to determine trophic level when thorough studies of foods from a particular ecosystem have been completed (Ambrose 1986, 1991; Cormie and Schwarcz 1996; Sealy et al. 1987).

Other studies have aimed to distinguish between marine and terrestrial diets based on differences in carbon and nitrogen isotopes of marine and terrestrial organisms, and have demonstrated that in most cases, marine foods tend to be enriched in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Chisolm et al. 1982; Schoeninger et al. 1983; Schoeninger and DeNiro 1984; Tauber 1981). In an early study to determine marine vs. terrestrial diet, Schoeninger et al. (1983) demonstrated that $\delta^{15}\text{N}$ values were 4‰ to 6‰ more positive in human groups that consumed marine organisms compared to agricultural groups. The authors studied precontact groups in North America, and South America, and pre-historic groups in Europe, but they noted that groups in the Bahamas had exceptionally low $\delta^{15}\text{N}$ values because they were consuming organisms from a coral-reef environment. Keegan and DeNiro (1988) addressed this anomaly in their paleodietary reconstruction for prehistoric Bahamians. Studying stable carbon and nitrogen isotopes in bone collagen, they noted that the nitrogen isotope ratios were dissimilar to values calculated for other coastal populations. They determined that marine resources from coral-reef communities resulted in enriched $\delta^{13}\text{C}$ and depleted $\delta^{15}\text{N}$ values with respect to other populations inhabiting a coastal environment. As with other paleodietary reconstructions, the capacity to differentiate between marine and terrestrial diets in past populations has allowed researchers to test the validity of preconceived notions of the foods certain groups consumed. Yesner et al. (2003) were able to revisit ethnohistoric and archaeological data compiled for populations of Tierra del Fuego using stable isotope analysis, demonstrating that the quantities of marine resources consumed by these groups differed prior to and after contact with Europeans, showing a shift in diet during a time of significant cultural/political change.

3.2.7 Investigations of African Slave Diets, Historical European Diets, and Military Diets

The non-segregated nature of the Royal Naval Hospital Cemetery (RNHC) in Antigua resulted in the recovery of individuals of both European descent and African or Afro-Caribbean descent. For this reason, it is essential in this chapter to provide dietary information for similar and contemporaneous populations. This section will focus on paleodietary reconstructions for enslaved labourers in both Africa and the West Indies, post-medieval Europeans, and colonial military personnel in both North America and Britain.

Several studies in South Africa have examined the remains of enslaved labourers (Cox et al. 2001; Cox and Sealy 1997). Cox and Sealy (1997) used stable isotope analysis of various skeletal elements formed at different stages of life and with different turnover rates to elucidate forced migration of enslaved labourers who died in a shipwreck off the coast of Cape Town. Cox and Sealy (1997) chose three different skeletal elements in order to study three separate life stages during which the dietary regime of the individual may have changed or remained the same. Isotopic ratios from tooth dentine, cortical bone, and trabecular bone are reflective of diet during early childhood, average diet over 15 to 20 years (Cox et al. 2001), and diet from the later years of life respectively. Stable isotope analysis thus permitted the investigation of changes in diet and residency during the lifetime of the individuals. Average $\delta^{13}\text{C}$ values for the individuals sampled were -11.5‰ for tooth dentine, -12.2‰ for cortical bone, and -16.2‰ for trabecular bone. These isotopic values demonstrated a general decrease in the consumption of C_4 foods during the lifetime of these individuals, but most substantially in the last part of their lives. Average $\delta^{15}\text{N}$ values for the individuals sampled were 8.4‰ in tooth dentine and 7‰ in cortical bone (no data were published for trabecular bone). These values suggested a decrease in animal protein between childhood and adulthood. In addition to dietary shifts during life, the authors were also able to examine evidence of dental modification to substantiate their estimation of each individual's origin. Isotopic analysis was thus useful in reconstructing life histories of these enslaved labourers who died en route to Brazil. Another study by Cox et al. (2001) was carried out on a colonial cemetery in Cape Town. Stable carbon and nitrogen isotope analysis permitted the authors to distinguish between local born and foreign born people. Those individuals with enriched $\delta^{13}\text{C}$ values consumed C_4 plants like maize, sorghum, or millet. When both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were enriched, this was indicative of consumption of marine resources. Depleted $\delta^{13}\text{C}$ values indicated consumption of C_3 plants, like wheat, oats, or rice. Individuals who had eaten C_4 foods in childhood were considered to be foreign born, while individuals who consumed a diet based on C_3 foods for their entire lifetime were considered to be local. Additionally, by combining stable isotope evidence with grave goods and physical traits (dental modification), the authors concluded that they could determine social status, including enslavement based on local vs. foreign diet.

Stable isotope analysis has also been carried out for enslaved populations in Barbados. In order to determine the origin of 25 enslaved Africans buried at Newton plantation, Schroeder et

al. (2009) studied stable carbon, nitrogen, oxygen (O), and strontium (Sr) isotopes in both bone and tooth dentine collagen (carbon and nitrogen) as well as tooth enamel (oxygen and strontium). The mean $\delta^{13}\text{C}$ value of both bone and tooth for the individuals tested was -10.0‰, while the mean $\delta^{15}\text{N}$ was 14.1‰, suggesting that the majority of the individuals consumed primarily C_4 crops and marine resources. The authors determined that most of the individuals had been born in Barbados rather than Africa, as demonstrated by little variation in their stable isotope signatures between different skeletal elements; however, seven individuals had stable oxygen and strontium isotope ratios that were inconsistent with birth in Barbados. Additionally, the stable carbon and nitrogen isotope ratios differed for these seven individuals between their teeth and bones, suggesting a change in diet that likely coincided with their enslavement. Based on the carbon, nitrogen, oxygen, and strontium stable isotope values, the authors narrowed down three regions in Africa from which these individuals likely originated before they were transported to the Caribbean.

Two other probable slave cemeteries from the Caribbean islands of Montserrat and Guadeloupe were sampled in order to conduct stable isotope analysis for dietary reconstruction (Varney 2003, 2011). The individuals buried at these cemeteries had stable carbon and nitrogen isotope ratios that were consistent with the expected slave diet outlined in historical sources, including consumption of primarily salt fish, with some salt beef or pork, as well as maize, yams, sorghum, sweet potatoes, plantains, and bananas (Dirks 1978; Dunn 1972; Luffman 1789; Varney 2003, 2011). Ranges for the stable isotope analysis were: $\delta^{13}\text{C}_{\text{collagen}}$ from -18.2‰ to -11.2‰, $\delta^{13}\text{C}_{\text{apatite}}$ from -7.6‰ to -5.4‰, and $\delta^{15}\text{N}$ from 11.1‰ to 17.9‰. (Varney 2003, 2011). Stable isotope analysis was also carried out for presumed enslaved individuals at the RNHC; this is covered in Chapter 4.

In a study of Medieval and post-Medieval diets in northeast England, Mays (1997) used stable carbon isotope signatures from collagen to determine the consumption of marine resources. Although most of the samples from this study dated from the tenth to the sixteenth centuries, one sample population of 10 individuals dated from the seventeenth to the nineteenth centuries. These individuals had a mean $\delta^{13}\text{C}$ value of -19.9‰, suggesting that they consumed a diet based on C_3 foods with a small contribution of marine resources. In another study of European foodways, a similar diet, consisting of C_3 plants, meat, and fish, was expected and found for individuals living in London in the mid-nineteenth century. Individuals local to

London were consuming a diet based on C₃ plants. Local Londoners could be distinguished from Irish migrants who had been consuming C₄ plants (maize had been imported to Ireland from the United States to lessen the effects of the famine; Beaumont 2013). These studies demonstrate, as was expected, that dietary staples in England from the seventeenth to nineteenth centuries were primarily C₃ plant products, meat, and some marine resources.

Stable isotope analysis was carried out for individuals interred in two naval hospital cemeteries from the late eighteenth/early nineteenth centuries in England, at Plymouth and Gosport (Roberts et al. 2012). This study is of particular interest since, like the RNHC, the populations represent individuals employed by the Navy and consuming naval rations for at least a portion of their lives. Additionally, the cemeteries in question are contemporaneous with the RNHC. Individuals buried at Plymouth and Gosport were expected to have had a relatively homogeneous “naval diet” consisting of primarily C₃ rather than C₄ staples, and terrestrial animals rather than marine resources. Mean $\delta^{13}\text{C}_{\text{collagen}}$ values for individuals interred at Plymouth and Gosport were -18.8‰ and -20.0‰ respectively. The mean $\delta^{15}\text{N}$ values were 11.1‰ and 11.9‰. The stable isotope ratios suggested that most individuals consumed a C₃ based diet with substantial quantities of terrestrial protein. However, at the Plymouth cemetery, some individuals had $\delta^{13}\text{C}$ values that indicated consumption of some C₄ products or animals fed with C₄ plants. Historical records from the hospitals indicated that more patients having served in the Americas and Caribbean were sent to Plymouth than to Gosport, which could account for some individuals at Plymouth having more positive $\delta^{13}\text{C}$ values than those at Gosport. Additionally, individuals originating from North America, either as prisoners-of-war or as volunteers for the British Navy, may also have had more positive $\delta^{13}\text{C}$ values due to the consumption of maize.

Another population of interest is that of soldiers of European and possibly Native American ancestry in North America. Soldiers from the United States killed during the War of 1812 were recovered in Fort Erie, Canada, and their remains sampled for stable isotope analysis. The mean $\delta^{13}\text{C}$ value for this group was -15.8‰ (ranging from ~ -12.5‰ to -18.5‰), demonstrating that staples consumed were both C₃ and C₄ grains. Nitrogen values for most of the individuals ranged from 9.6‰ to 11.8‰, although some individuals had $\delta^{15}\text{N}$ values as high as 13.0‰. The higher $\delta^{15}\text{N}$ values suggested consumption of a diet high in meat and/or fish (Katzenberg 1991). The same sample of individuals was later re-studied, revealing similar results

to the initial investigation. The dietary reconstruction for these soldiers was more reflective of their diet prior to joining the army rather than during their time as soldiers, given that these individuals were likely recruited only months before they died at Fort Erie. Thus, the amount of heterogeneity found in the stable isotope signatures for this population was not surprising (Raynor and Kennett 2008).

These studies confirm that individuals living in Europe tended to rely primarily on C₃ foodstuffs rather than C₄ products that would not have been widely available or desirable during the eighteenth and early nineteenth centuries. In contrast, individuals of African ancestry in transit to the New World or already living there typically consumed C₄ plants as part of their basic dietary staples. Individuals of European ancestry living in the United States also consumed C₄ plants to varying degrees. Stable nitrogen isotope signatures demonstrate that marine resources were of varying importance for populations of both African and European ancestries.

3.3 Trace Element Analysis and Studies of Bone Lead Levels

3.3.1 Introduction to Trace Element Analysis

Trace element analysis of human remains originated in the 1970s as a method to directly reconstruct past human diets (Klepinger 1984; Sandford and Weaver 2000). The quantification of certain elements was perceived to be a method of reconstructing prehistoric diets, specifically the proportion of meat and plant resources, and applying the results to archaeological questions related to health and disease, status based on social constructs, and subsistence (Blakeley 1977). Despite the initial faith in trace element studies, it quickly became apparent that diagenesis could have a considerable impact on the reliability of trace element analysis (Radosevich 1993). Additional complications arose from misconceptions surrounding the physiological basis for inclusion of certain elements as indicators of diet (Ezzo 1994). This section presents a brief overview of general trace element studies and their limitations, followed by a summary of lead trace element analysis, including past contributions to archaeological research on lead, limitations of past research, new technologies that allow for continued research, the physiological incorporation of lead into the body, and its effects on health in humans.

3.3.2 A Brief Overview of Trace Element Analysis

Researchers applied the methods of trace element analysis to archaeology hoping to utilize the quantities of trace elements in samples of bone from humans and fauna for

paleodietary reconstruction in order to determine the proportions of meat and plant resources consumed by past human populations (Gilbert 1977; Sandford and Weaver 2000). Trace element analysis, at the time, was considered to be a great advancement in archaeological techniques because it provided a direct indication of human diet, rather than an indirect one obtained through the analysis of refuse at human occupation sites (Sandford 1993). Trace elements are those that occur naturally, except for bulk elements and macrominerals, and in very small quantities. Bulk elements include nitrogen, carbon, sulfur (S), oxygen, and hydrogen (H), while macrominerals include sodium (Na), magnesium (Mg), phosphorus (P), chlorine (Cl), potassium (K), and calcium (Ca) (Mertz 1981). Trace elements may be essential to the life of an organism, involved in the functioning of biological systems when maintained at the required levels. Trace elements can also be nonessential, and some, including lead, mercury (Hg), and cadmium (Cd), can have detrimental effects on the biological systems of an organism even at low doses. All trace elements, essential and nonessential, can be toxic in excessive quantities (Apostoli 2002).

Trace elements can be incorporated into the body through dietary intake or environmental exposures during life. The body, through homeostatic mechanisms, works to control trace element concentrations in the tissues (Parker and Toots 1980). An organism's metabolic processes have an effect on the quantities of elements in the body through absorption, excretion, pregnancy, lactation, and growth (Sandford and Weaver 2000). Thus, the body regulates the concentrations of many trace elements through a variety of mechanisms to maintain homeostasis. Incorporation of trace elements into the bone can happen in a variety of ways. Spadaro et al. (1970) noted that certain trace elements, such as copper (Cu), iron (Fe), and zinc (Zn), are located in the collagenous component of bone, while most trace elements, including lead, silicon (Si), strontium, and vanadium (V), are located in the mineral component of bone. Zinc can be divided between both collagenous and mineral components of bone. Recent research has demonstrated that lead can be taken up directly by the hydroxyapatite, but also by attaching itself to non-collagenous proteins, which have an affinity for lead (Pemmer et al. 2013). As an example of how a trace element can be incorporated into bone, strontium, a nonessential trace element (Ezzo 1994), can be taken up as part of the diet, and incorporated into bone by two different mechanisms. Strontium enters into either direct surface exchange or ionic substitution with calcium atoms up to a maximum of one strontium atom per 10 calcium atoms. In direct surface exchange, the surface of bone in contact with blood quickly exchanges calcium for

strontium. In ionic substitution, or diffuse exchange, the entire bone is penetrated and exchange occurs slowly between strontium and calcium. Strontium levels in bone mineral reach a plateau regardless of increasing quantities of strontium in the diet, because only a certain percentage of calcium can be exchanged for strontium (Dahl et al. 2001). Other trace elements are also incorporated into bone via ionic substitution and result in the replacement of ions in the hydroxyapatite (which contains calcium ions) and the alteration of the hydroxyapatite's normal chemical composition. A variety of different exchanges can occur, including the exchange of strontium, lead, magnesium, or sodium ions for calcium ions (Neuman 1980).

Sandford (1993) categorized trace element studies into three major applications based on the element of interest: strontium and strontium/calcium (Sr/Ca) analysis, multielement analysis, and single-element analysis. Early studies that fell within the first category determined concentrations of strontium and/or Sr/Ca ratios in order to deduce the relative importance of plants foods vs. animal proteins in the diet (Gilbert 1977). Strontium moves from soil to the human body via the food chain (Comar et al. 1957). Plants do not discriminate between strontium and calcium; therefore their Sr/Ca ratio reflects that of surface water. However, when plants are ingested by animals, calcium is favoured over strontium. Most of the strontium ingested is excreted, but some is absorbed through the gut into the blood, and is finally incorporated into bone using one of the mechanisms described above. Strontium is thus absorbed in quantities that indicate an organism's position in the food chain, with carnivores having smaller quantities of strontium than herbivores, as strontium is incorporated into the body tissues through the diet (Comar et al. 1957; Sandford 1992). Studies of strontium were a popular area of investigation in trace element analysis in order to determine the proportion of animal and plant resources in the diet through comparison of bone samples from humans and animals from the same geographical area (Klepinger 1984). Initially the use of strontium to reconstruct diet seemed to hold promise and was applied to studies of the development of agriculture in the Middle East (Schoeninger 1981), social stratification in a precontact population in Georgia, United States (Blakely and Beck 1981), the estimation of age of weaning for prehistoric groups (Sillen and Smith 1984), and other areas of archaeological interest.

Strontium, specifically, posed some problems for interpretation because it was initially supposed to represent the relative contribution of plants to the diet. For instance, Schoeninger and Peebles (1981) found that the consumption of molluscs increased the level of strontium in

the bone and thus, without the available archaeological data for consumption of molluscs, the interpretation of diet would have been biased for this population. Additional limitations of strontium trace element studies included the difficulties with which inter-site comparisons could be made. Sillen (1992) found that strontium concentrations varied in different regional food webs due to differing geological characteristics of an area. Foods within a trophic level (i.e. different plant types) could also vary in their strontium concentrations. Thus, a good understanding of the foodwebs in a particular area is needed before any interpretations of strontium concentrations can be carried out (Sandford and Weaver 2000). Additional limitations of strontium and other trace element analyses are discussed below.

Multielement analysis quantified a variety of different elements in order to reconstruct past diet. Certain elements were considered to be useful in determining the contributions of plant and animal resources to the diet (Hatch and Geidel 1985). Specifically manganese (Mn), magnesium, strontium, and vanadium were thought to be useful to determine the extent to which plant resources were exploited, while copper, zinc, selenium (Se), and molybdenum (Mo) were used to determine the extent to which animal resources were exploited (Sandford 1993; Schroeder et al. 1963, 1966a, 1966b, 1972). Vanadium, for example, is found in much higher concentrations in plant foods than in meats (Hatch and Geidel 1985; Schroeder 1963), while zinc tends to be found in higher concentrations in meats (Ezzo 1994; Hatch and Geidel 1985). Various studies used analysis of multiple elements to study variation in diet related to status and gender (Blakely and Beck 1981; Hatch and Geidel 1985), and focused on subsistence shifts in past populations, particularly the shift from hunting and gathering to horticulture (Beck 1985; Buikstra et al. 1989). Despite the authors' various discussions of the potential limitations of multielement analysis due to diagenesis and the effects that changes in the body, such as age or pregnancy, can have on elemental concentrations, the investigators carried on with their studies conceding that there were various unknown factors that could have an effect on their results (Beck 1985; Blakely and Beck 1981; Hatch and Geidel 1985).

Single-element analysis typically approached questions that related diet to disease in a population. A major area of interest within single-element analysis was the study of lead, a toxic element, to which a variety of populations have been exposed (Sandford 1993). Iron (Fe) was another element of interest in single-element analysis due to the presumed relationship between iron-deficiency anemia and the manifestation of cribra orbitalia on bone (Fornaciari et al. 1983).

Lead trace element analysis is explored in greater detail below, as this is a major area of focus of this thesis.

3.3.3 Critiques of Trace Element Analysis

Although some difficulties related to the limitations of trace element analysis had been raised by authors doing research in the area (Beck 1985; Blakely and Beck 1981; Hatch and Geidel 1985), the lack of appropriate responses to the limitations resulted in several critiques of trace element analysis in the early 1990s (Ezzo 1994; Radosevich 1993). Radosevich (1993) concentrated on what he called the six deadly sins of trace element analysis, and stated that they were assumptions about bone geochemistry that were made by the majority of researchers doing trace element analysis. The two most critical “sins” were the assumptions that strontium and zinc, specifically, are not affected by diagenesis, except under unusual circumstances, and that diagenesis can be detected through soil analysis, based on the belief that if quantification of trace elements in bone is different than in soil there has been no exchange of elements between the two materials (Radosevich 1993). These types of assumptions have been and continue to be detrimental to archaeological research because they allow researchers to uncritically interpret data from bones that were probably affected by diagenetic processes in the postmortem environment.

Radosevich’s (1993) critiques focused on the diagenetic limitations of trace element analysis, while Ezzo’s (1994) critiques focused on the usefulness of certain elements for paleodietary reconstruction based on physiology and mineral metabolism. Through the establishment of several criteria to create a model for using trace elements as dietary indicators, Ezzo (1994) suggested that only strontium and barium (Ba) are useful for the reconstruction of paleodiets. His suggested criteria for indicators of diet include: 1) the element in question should concentrate in bone and be incorporated into the hydroxyapatite crystal rather than remain on the surface of the bone; 2) the element must be a direct reflection of diet; 3) the element should be a nonessential element that mimics the movement of essential elements. Ezzo (1994) stated that he established these criteria because of the need for an understanding of the physiological basis for any trace element in bone before it could be used as an indicator of diet. He claimed that trace element analysis had been misinterpreted because, for many elements, it was not possible to establish this physiological basis. Based on the critiques presented by both Ezzo (1994) and Radosevich (1993), there have been many strong arguments for the complete reevaluation of

many trace element studies, whether due to the inappropriate elements chosen to reconstruct diet or due to diagenetic factors that are likely to have affected the elemental concentrations within bone.

3.3.4 Recent Investigations of Trace Elements

More recent investigations have included both barium and strontium in their analysis (Burton and Price 1990; Ezzo 1992; Gilbert et al. 1994). Barium was found to be similar to strontium in that its concentration in bone varies inversely to the trophic level of the organism because barium is discriminated against in favor of calcium as organisms move up the food chain (Elias et al. 1982). Through studies of barium and strontium concentrations in bone, researchers have been able to look at terrestrial vs. marine diets in coastal and inland populations (Burton and Price 1990; Gilbert et al. 1994), as well as faunal trophic levels and diagenesis in a desert environment (Ezzo 1992). Ba/Sr ratios provide an indication of marine diets because seawater has a very low Ba/Sr ratio in comparison to terrestrial environments (Burton and Price 1990).

With regards to the other trace elements that Ezzo (1994) demonstrated to be ineffectual as paleodietary indicators, it might seem prudent to completely eliminate them from archaeological analysis altogether. However, Burton (2008) suggested that if they were completely ignored in trace element analysis, additional information would be lost, such as using these trace elements as diagenetic indicators. Ezzo (1994) and Price (1989) noted that elements that are not biologically incorporated into the hydroxyapatite, although not indicative of diet, can be used as indicators of diagenetic processes. Therefore, they argued that the continued use of multielement analysis can certainly be beneficial to archaeological research. Shafer et al. (2008) addressed diagenesis using trace element analysis by applying sequential acid leaching to bone samples and comparing metal levels on the surface of the bone to metal levels from an interior core taken from compact bone. They were able to estimate the degree to which the surface of the bone had been enriched in certain trace elements in comparison to the bone core, indicating the extent of contamination. However, using this method, they could not quantify how much contamination had occurred within the bone, rather only that which had remained on the surface of the bone.

Recently there have been substantially fewer trace element studies seeking to reconstruct diets for past populations from skeletal remains because of the greater efficacy of stable isotope analysis (Burton 2008). However, strontium continues to be used to infer past dietary habits from

skeletal remains and cremains (Gonzalez-Reimers et al. 2001; Janos et al. 2011; Szostek et al. 2003). Additionally, to avoid problems of diagenesis common in bone, recent trace element studies have used tooth enamel because of its increased resistance to environmental contamination (Brown et al. 2002; Cucina et al. 2011). Focus has also shifted away from dietary reconstruction to other uses for trace element analysis. As previously mentioned, trace elements may be used to study diagenetic effects on bone (Janos et al. 2011; Shafer et al. 2008), but they are also used to determine population origins and migration from tooth enamel (Brown et al. 2002; Cucina et al. 2011).

Although there was initial enthusiasm in the potential for trace element analysis to be a direct indicator of paleodiet, a variety of challenges arose over time that complicated the issue. However, trace element analysis has not been entirely abandoned, as lead trace element analysis continues to be a part of archaeological research and will be the focus of the remainder of this chapter.

3.3.5 Trace Element Analysis of Lead in Archaeological Research

In vertebrates, lead acts much like strontium and barium. It tends to accumulate in bone as a result of replacing calcium in the hydroxyapatite and follows biopurification processes at increasing trophic levels (Elias et al. 1982). Lead is considered to be a general metabolic toxin that, even at low levels in the body, can cause poisoning in both humans and animals (Bellinger and Matthews 1998; Damstra 1977). Archaeological studies of lead in human bone have focused on questions of lead toxicity in past populations (e.g. Corruccini et al. 1987; Drasch 1982; Jarcho 1964; Keenleyside et al. 1996, 1997; Kjaer et al. 2009; Kowal et al. 1989; Reinhard and Ghazi 1992), as well as the determination of socioeconomic differences and access to contaminated goods or occupational exposure (Aufderheide et al. 1981; Aufderheide et al. 1988; Reinhard and Ghazi 1992), and the birthplace of individuals who may have endured forced migration (Schroeder et al. 2013). Additionally, lead isotopes have been studied to identify the source of lead in some cases of exposure (Ghazi et al. 1994; Kowal et al. 1991; Reinhard and Ghazi 1992). A great deal of attention has been paid to the differentiation of diagenetic vs. biogenic lead in skeletal remains, in order to ensure that the concentrations being studied represent lifetime exposure to this toxin, rather than contamination from the burial environment (Ghazi et al. 1994; Reinhard and Ghazi 1992; Waldron 1981, 1983; Wittmers et al. 2008).

Determining the extent of lead toxicity in archaeological remains is a complicated matter due to the ways in which lead is incorporated into the tissues of the body. It may be ingested, absorbed, or inhaled and thus enters into the blood stream, the soft tissues, and the skeleton. Though lead remains only a short period of time in the blood and soft tissues, it has a half-life of years to decades in bone and is considered to represent a lifetime of accumulation in an individual (Brodkin et al. 2007; Rabinowitz et al. 1976). Clinical literature that associates blood lead levels with symptoms of lead poisoning (Brodkin et al. 2007) does not provide the same information for bone lead levels. A study by Somervaille et al. (1988) related bone lead levels to blood lead levels in occupationally exposed workers in the United Kingdom (UK). Although they found high correlation between blood and bone lead levels and developed a least squares regression equation ($\text{Tibia Pb } [\mu\text{g/g wet bone}] = 0.0296 [\pm 0.0015] \times [\text{time integrated blood Pb index}] - 2.285 [\pm 1.102]$) to determine cumulative blood lead levels from bone lead levels, the investigators did have some concerns regarding missing blood lead levels from early in the workers' careers. Correlation between blood and bone lead levels has been adapted for use in archaeological investigations to determine whether individuals suffered symptoms of lead poisoning or whether the lead levels were low enough that the individual had subclinical exposure (Corruccini et al. 1987; Handler et al. 1986; Keenleyside et al. 1996, 1997). The conversion to blood lead levels from bone lead levels permits the estimation of potential symptoms and severity of intoxication, which would be difficult without this conversion. However, due to the correspondence of bone lead levels to a lifetime of exposure, and blood lead levels to acute exposure (Brodkin et al. 2007; Rabinowitz et al. 1977), there remains some question as to the accuracy of these conversions (Corruccini et al. 1987).

In other archaeological investigations, lead toxicity has been speculated based on high levels of lead in the bone and historical documentation of exposure via contaminated goods or archaeological evidence of contaminated goods (Kjaer et al. 2009; Kowal et al. 1989; Reinhard and Ghazi 1992). In the case of the mid-nineteenth century Franklin Expedition, one study was able to take samples from soft tissues of some of the deceased and found elevated lead levels in the soft tissues. Given the short time period during which lead remains in the soft tissues during life, the authors felt that exposure had taken place during the expedition (Kowal et al. 1991). Several studies detailing lead exposure in both bone and soft tissues for the Franklin Expedition have been criticized for their assumptions that the lead burden in the individuals was the result of

exposure that occurred during the expedition (Farrer 1993). Again, due to the physiological incorporation of this element into the body tissues, it is difficult to determine, even with the soft tissues of the deceased, whether the lead exposure occurred only during the expedition, or if there had been constant long-term exposure prior to the expedition. Farrer (1993) noted that in nineteenth century Britain, lead exposure was common through a variety of sources including food and water, and that the background levels of lead in individuals from this time period would have been quite high. Additionally, Farrer (1993) points out the need for a comparison between lead levels in the sailors of the Franklin Expedition with contemporaneous British males to determine whether the lead levels were indeed higher than normal for that time period.

Background levels of lead in human remains from populations that were unexposed to anthropogenic lead sources during life have been studied in order to determine “natural” levels of lead in bone in the human body (Drasch 1982; Patterson et al. 1991). Natural levels of lead cannot be determined from modern individuals because of environmental contamination in both industrialized and non-industrialized geographical areas (Bellinger and Matthews 1998). Therefore, in addition to analysis of archaeological populations that fit the criteria of not having been exposed during life, calculations, using simple linear regression, have also been carried out to determine natural blood lead levels in humans (Flegal and Smith 1992). This work is essential because it allows researchers to determine when lead levels in human remains are above what occurs naturally, or whether the levels fall within the range of naturally occurring lead from the pre-industrial environment. Although this knowledge cannot assist in the determination of whether individuals suffered symptoms of lead poisoning, it certainly assists researchers in understanding the substantial increase in body lead burden that the cultural usage of lead has caused over time.

Another application of lead trace element analysis in archaeology is to distinguish between different socioeconomic groups in a population. Occupational exposure is one way in which individuals may have accumulated lead in their bones, but they could also have been exposed through use of certain goods and foodstuffs (Aronson 1983; Aufderheide et al. 1981; Aufderheide et al. 1992; McCord 1953a, 1953b). In a Colonial American population, Aufderheide et al. (1981, 1988) were able to use bone lead levels as indicators of socioeconomic status on a plantation. Because wealthy white owners had access to food containers that were lead-lined, they had much higher lead levels than the black enslaved labourers and white

indentured servants buried at the plantation. One black female had higher levels of lead, which suggested that she was occupationally exposed through work as a domestic in the owner's household. In stark contrast to the low levels of lead in the enslaved population in the Colonial American cemetery, in the Colonial Caribbean, Barbadian plantation enslaved labourers had extremely high levels of lead in their bones (Handler et al. 1986). These archaeological investigations distinguish between socioeconomic groups and demonstrate exposure to lead through occupation or access to contaminated goods and foodstuffs, providing noteworthy historical information on past lifeways, and demonstrating how exposure to lead was drastically dissimilar in different geographical locations. Additionally, these investigations have provided indications as to past occupations, such as domestic service or sugarcane processing, which resulted in substantially different bone lead levels than the majority of people in the same socioeconomic or ancestral group.

The differentiation between socioeconomic groups incorporates the question of access to contaminated goods and occupational exposure. The avenues of exposure have also been investigated without the objective of determining differences between socioeconomic groups. Reinhard and Ghazi (1992) studied the lead content in skeletal remains from two eighteenth century Omaha Indian cemeteries and found that all the individuals had above natural levels of lead in the bone. Although they demonstrated, through artifactual evidence, that the lead exposure could have been as a result of trade with Euroamericans, as well as occupationally through manufacture of lead goods, a major limitation of this study was that, in several cases, extremely high levels of lead were recorded. These abnormally high concentrations were considered to be due to a cultural practice of applying coloured lead pigments to the deceased, and it was not possible to separate the biogenic lead from the culturally introduced diagenetic lead. This demonstrates the difficulty with which archaeologists interpret the source of both biogenic and postmortem diagenetic lead when various sources are possible. In another study, it was not so much the specific source of the lead that was of note, but the presence of lead at all in a study to determine the origins of Barbadian plantation enslaved labourers (Schroeder et al. 2013). In this study, tooth enamel, which forms early in life, was used instead of bone to determine whether the individuals had been exposed during the first few years of life. Those who had not been exposed to lead during tooth formation were likely of African origin, while those who were exposed were likely born in Barbados.

3.3.6 Diagenesis

A major concern for archaeologists using lead trace element analysis is diagenesis. In buried bone there is always the possibility of postmortem processes either adding or reducing the lead content in the hydroxyapatite of bone (Wittmers et al. 2002). In the case of the Franklin Expedition, diagenesis was not considered to be a factor affecting the skeletal remains because the bones were found as a surface scatter, and comparison of caribou bone and Inuit bone from the area showed low lead levels, indicating that the Arctic environment likely did not contribute to the lead burden in the remains of the sailors (Kowal et al. 1989). In most cases, however, human remains have been buried in sediment and thus diagenesis may have played a more substantial role in changing the chemical composition of the bones. This was a considerable problem at the early nineteenth century First African Baptist Church burial ground, in Philadelphia, where high levels of lead in the remains were associated with a diagenetic origin (Wittmers et al. 2008). A variety of different reasons for high lead levels were examined by the authors, who found that poor histological structural preservation of the bone contributed to increased contamination from the burial environment. Children also had high lead levels because of the porosity of their bones (Wittmers et al. 2008). Another case of high levels of lead attributed to diagenesis occurred when individuals in two British cemeteries were buried in lead coffins, which promoted postmortem lead contamination on the surface of the bones (Waldron 1981). In cases where a large quantity of lead has been deposited in the bones postmortem, it is impossible to separate the biogenic from the diagenetic lead to determine the actual exposure during the lives of the individuals. This issue makes such studies of contaminated bones limited in terms of what can be learned about exposure during life, but can be useful in studying postmortem processes that have an effect on bone (Waldron 1981; Wittmers et al. 2008). Waldron (1983) detected high levels of lead in the remains from a Cistercian monastery and from a Romano-British site. He concluded that, despite low levels of lead in the surrounding burial environment, diagenesis had taken place and elevated the lead levels in the bones and teeth of the individuals. He determined this based on statistical analysis which suggested that the lead levels in bone were significantly correlated with the lead levels in the sediment. Though an early study, this article presented a cautionary tale for investigators that low lead levels in sediment are not necessarily indicative of low possibility for diagenesis. Another important aspect of this study is that it is an early example of the investigation of the spatial distribution of lead, in this

case in a tooth. This was carried out to determine whether the distribution of the lead was indicative of a diagenetic or biogenic origin. Because the lead was concentrated on the external surface of the tooth rather than primarily in the circum-pulpal dentine, Waldron (1983) considered that the lead was due to postmortem contamination. More recent technologies are now also used to assess spatial distribution of lead in bone for this same purpose, and are further discussed in section 3.3.7.

Efforts have been made to minimize the effects of diagenesis on bone for trace element investigations (Wittmers et al. 2002). Lambert et al. (1991) suggested the removal of 1mm of the surface of bones to remove contaminants, while Sillen (1986) suggested the immersion of the bone in a sodium acetate solution of pH 4.5 because he found that hydroxyapatite formed in the burial environment would be dissolved while biological apatite would be unaffected by the solution. Despite these potential solutions for diagenetic effects on bone for trace elements in general, Wittmers et al. (2008) demonstrated, by their inability to distinguish between biogenic and diagenetic lead in the First African Baptist Church burial ground, that in cases of severe postmortem contamination, the simple mechanical cleaning of the surface of bone and the use of an infiltrating solution are not sufficiently reliable methods of correcting diagenetic effects. Thus, it is essential to determine the extent of diagenesis on bone before determining whether an interpretation of biogenic lead exposure is possible or not.

3.3.7 Technological Innovations Used to Address Questions of Diagenesis in Bone

Recently, synchrotron x-ray fluorescence has been used to map the spatial distribution of lead in bone (Martin et al. 2007, 2013; Pemmer et al. 2013; Swanston et al. 2012; Wittmers et al. 2008). The earliest of these studies examined lead distribution in a bone and tooth sample from a pre-Columbian burial from Peru. The lead was concentrated near the periosteum of the bone and in the cementum of the tooth. Although the authors felt that the individual had been exposed to lead during life, they did not feel they had sufficient evidence to be certain of the lead's biogenic origins (Martin et al. 2007). Another study, though not providing an indication of the exact location of the lead within the bone microstructure, demonstrated that the lead in the remains from the First African Baptist Church followed three patterns: higher levels of lead near the periosteal surface, near the endosteal surface, or in an irregular pattern between the surfaces. The authors felt that higher lead levels either at the periosteal or endosteal surfaces were indicative of

diagenetic lead. In contrast, they felt that the irregular distribution could indicate either physiological or diagenetic lead (Wittmers et al. 2008). In both these studies the authors were unable to distinguish between biogenic and diagenetic lead exposure. Swanston et al. (2012) were able to map lead in the bone microstructure of a sample from an individual buried at the RNHC. They found that the irregular labeling of canal surfaces and surface lamellae suggested biogenic uptake of lead. It was, therefore, necessary to examine precisely where the lead had been taken up into the bone to determine its origin as either biogenic or diagenetic. Similar synchrotron analyses were carried out on skeletal material from the Franklin Expedition to determine whether there had been a sudden increase in lead levels during the last years of the individuals' lives. Through mapping of lead across the surface of a section of bone, the authors concluded that the lead was biogenic, but that the uptake of lead had occurred as a result of long-term exposure rather than an acute episode during the expedition. This was determined based on both the wide distribution and high concentrations of lead in the bones tested (Martin et al. 2013). Although the earliest use of synchrotron x-ray fluorescence to examine the distribution of lead in bone did not result in sufficient evidence to distinguish between biogenic and diagenetic lead uptake, more recent studies that have looked at the precise location of lead in the microstructure of the bone have led to more confidence in the capacity to determine whether exposure occurred during life or in the postmortem environment.

3.4 Lead Incorporation into Bone and its Health Effects

3.4.1 Lead Incorporation into Bone

Environmental lead can be absorbed, ingested, or inhaled into the body. It is then absorbed into the blood and transported throughout the body, 90% being excreted. Of the remaining 10% of lead, 95% is incorporated into the skeleton, primarily into the cortical bone (Barry 1975). Lead has a relatively short half-life in both the blood and soft tissues, measurable in weeks, but has a very long half-life in bones, remaining in the skeleton for years to decades (Brodkin et al. 2007; Rabinowitz et al. 1976). Due to the long period of time during which the lead remains in bone, bone lead levels are considered to be indicative of the average lifetime lead exposure (Aufderheide 1989). Lead is incorporated into bone microstructure where there is active calcification during the remodeling process. Osteons, the main components of bone microstructure, are labeled with lead while they are in the process of forming (Aufderheide and

Wittmers 1992). Lead tends to accumulate to a greater extent at the cement lines, which connect individual osteons (Pemmer et al. 2013). During the remodeling process, osteons can also undergo lysis, at which point lead is released back into the blood stream to be excreted or re-deposited in newly forming osteons (Aufderheide and Wittmers 1992). The way in which lead is incorporated into bone changes as a result of the age of an individual, with children and adults experiencing differential deposition of lead in bones due to different areas of active growth or remodeling. For example, children tend to accumulate more lead in the ends of long bones, due to the concentrated areas of growth in these bones, rather than in the diaphyses (Aufderheide 1989). Thus, in order to interpret lead burden in past populations, consideration of the variety of factors that affect the physiological incorporation of the element into the body is necessary, as not all bones or portions of bones will contain the same quantities of lead, and the skeletal burden is not reflective of acute lead exposure, but rather, as previously noted, long-term exposure from various sources.

3.4.2 Health Effects of Lead

Lead has no apparent function in the human body, and in fact, is toxic to most organs (Aufderheide 1989). Acute lead poisoning manifests itself with symptoms of anemia, headache, colic, fatigue, abdominal pain, muscle pain, and peripheral neuropathy in adults (Brodkin 2007; Damstra 1977). In children, lead poisoning results in vomiting, anorexia, convulsions, and encephalopathy (Damstra 1977). In extreme cases, lead poisoning may result in coma or even death (World Health Organization (WHO) 2014). Lead primarily affects the renal, hematopoietic, gastrointestinal, and nervous systems (Aufderheide 1989; Damstra 1977). Though these symptoms are associated with acute lead poisoning, there is evidence that continuous exposure to low lead levels is also detrimental to an individual's health, as exemplified by problems in brain development in children (Weiss 1990). Given that the standards for a safe quantity of lead intake are based on body weight and that observations of differential tolerance to lead have been made between the sexes (Mahaffey 1977), there are a variety of factors of an individual nature that affect the way lead is taken up in the body. In an archaeological context, therefore, it is with great difficulty that the researcher is able to assess the health impact of lead exposure on an individual. However, in cases with sufficient historical documentation of lead poisoning coupled with high levels of lead in the remains of individuals from that population

(Handler et al. 1986), it can be assumed that some individuals did indeed suffer symptoms of lead toxicity consistent with clinical literature on the effects of exposure. The potential health effects due to lead exposure for the individuals recovered from the RNHC are presented in detail in Chapter 6.

3.5 Summary

Archaeological bone chemistry, including both stable isotope analysis and trace element analysis, is a valuable resource used in reconstructing past lifeways. These analyses can provide information on dietary regimes, differences in social status and gender, access to goods, as well as health and nutrition.

Stable isotope analysis has been used for decades in the reconstruction of paleodiets from carbon and nitrogen stable isotopes. These studies have provided the means to distinguish between diets based on C₃ or C₄ plant foods, marine vs. terrestrial diets, and trophic level. Although there are many challenges involved in the interpretation of stable isotopes for dietary reconstruction in many geographical areas of the world, the establishment of possible foods consumed in a region and time period, and knowledge of the climate and geography of an area can be used to interpret data more clearly.

Trace element analysis, as it has been used to reconstruct diet, has become less popular among archaeologists due to the complications of diagenesis, although certain studies involving strontium and barium as paleodietary indicators are still conducted. Trace element analysis of lead has also continued to be carried out, especially in light of new technologies that permit researchers to distinguish more confidently between biogenic and diagenetic lead.

Lead is incorporated into the microstructure of bone and is representative of long-term exposure due to the long half-life of lead in the skeletal tissues. It is toxic to humans and has a variety of ill effects on an individual's health. In the most severe cases, it can result in paralysis and even death.

Stable isotope analysis and trace element analysis carried out for the individuals interred at the RNHC are presented in Chapter 4, along with details of the archaeological excavation and analysis of the skeletal remains.

Chapter 4: Materials and Methods

4.1 Introduction

This chapter presents an overview of the materials and methods used to conduct this research. This thesis focuses on analysis of previously collected data from the skeletal remains recovered from the Royal Naval Hospital Cemetery (RNHC), English Harbour, in Antigua, West Indies. This chapter includes a brief history of the excavations and skeletal analysis of individuals buried at the RNHC, as well as stable isotope and trace element analysis previously carried out for these individuals. Methods used for the analysis and interpretation of the data, including statistical analyses, are presented. Methods used to quantify lead levels in the skeletal remains of the individuals from the RNHC are also covered.

4.2 Materials

4.2.1 Excavations at the Royal Naval Hospital Cemetery

The RNHC is a unique cemetery site in the West Indian colonial context because it is the only known, excavated, non-segregated cemetery, containing the remains of those of both African and European descent (Varney 2003, 2011). It is also the only naval hospital cemetery to be excavated in the West Indies. The RNHC was in use from A.D. 1793–1822, and associated with the Royal Naval Hospital at English Harbour. Due to modern urban development, much of the cemetery had been disturbed. Archaeological excavations of undisturbed portions of the cemetery ran from 1997–2001 (Varney and Nicholson 2001). The single remaining grave marker from the site was preserved; it bears the name of the assistant ship's surgeon of the *H.M.S. Pyramus*, Alexander Bernard. Bernard died in 1821 at the age of 27 years (Nicholson 1995; Varney and Nicholson 2001). All other individuals buried at the RNHC are of unknown identity due to the absence of inscribed tombstones and the scarcity of historical documentation referring to the hospital and burial grounds (Varney and Nicholson 2001).

A total of 30 individuals were recovered from 26 graves excavated at the RNHC. Some graves were found to be overlapping with, and causing disturbance of, earlier graves. This is suggestive of intensive use of the cemetery. The majority of the graves contained single burials.

However, some graves were sequential interments, with more than one individual buried at different times, and one grave contained a double inhumation. The recovered individuals consisted of 21 adults, and nine subadults. Of the subadults, five were under the age of five years. Individuals were most commonly buried in simple wooden coffins; however, five burials showed no evidence of a coffin. Associated objects recovered from the adult burials were few and consisted primarily of buttons of wood, shell, or metal, and two buckles. Associated objects recovered from the subadult burials were either shroud pins or beads located near the neck (Varney and Nicholson 2001; Varney 2003, 2011).

Osteological analysis was carried out according to standards established for data collection from human remains (Buikstra and Ubelaker 1994; Varney 2003, 2011). When sex could be estimated for the recovered individuals, it was determined to be male (Varney 2003, 2011). Ancestry for adult and adolescent individuals was estimated based on cranio-facial features according to forensic anthropological standards (Gill and Gilbert 1990; Rhine 1990; Varney 2003, 2011). Seven individuals were estimated to be of European or white ancestry, and seven individuals were estimated to be of African or black ancestry. Individuals with damaged or missing cranio-facial features could not be analyzed for ancestry. Table 4.1 contains summary information on the individuals recovered from the RNHC.

4.2.2 Dietary Reconstruction via Stable Isotope Analysis

Stable isotope analysis has provided information on the diets of all individuals excavated from the RNHC. Dietary reconstruction from stable carbon and nitrogen isotopes revealed both similarities and differences in diet between those of African and those of European descent. It is most likely that individuals of African ancestry were enslaved labourers owned by the British Navy, while those of European descent were lower-ranking naval personnel (Nicholson 2002; Varney 2003, 2011). Higher-ranking naval personnel were commonly buried in parish cemeteries, and plantation enslaved labourers working at the dockyard were likely buried by the owner rather than the Navy (Varney 2003). Both bone collagen and bone apatite samples were analyzed for stable isotopes, as they provide different information on diet (Varney 2003, 2011). Bone collagen reflects the protein in diet through analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, whereas bone apatite reflects the diet as a whole through analysis of carbon (Ambrose and Norr 1993).

Table 4.1 Summary of Individuals Recovered from the RNHC*

Individual	Ancestry	Age	Sex	Year excavated
B1	A	45–49	M	1997
B2	E	25–29	M	1998
B3	E	30–34	M	1998
B4a	A	50–60	M	1998
B4b	U	Newborn	I	1998
B5	E	16–18	M	1998
B6	E	25–29	M	1998
B8-1	U	14–15	I	1998
B8-2	U	≥20	I	1998
B8-3	U	20–29	I	1998
B9a	U	18–20	M	1998
B9b	U	20–29	I	1998
B12a	A	35–39	M	1999
B12b	U	2–4	I	1999
B13	A	45–49	M	1999
B14	U	35–39	M	1999
B15a	E	35–39	M	1999
B15b	U	Newborn	I	1999
B16	U	16–18	M	2000
B17	U	35–39	M	2000
B18	E	30–35	M	1999
B19a	E	40–45	M	1999
B19b	U	20–24	M	2000
B20	U	1–1.5	I	2000
B21	U	0–0.5	I	2000
B22	U	20–29	M	2001
B23	A	35–39	M	2001
B24	A	25–29	M	2001
B25	A	20–25	M	2001
B26	U	14–18	M	2001

* Data from Varney (2003); Ancestry: A (African), E (European), U (unknown); Age: years of age at death unless otherwise indicated; Sex: M (male), I (indeterminate).

Based on historical sources, it was expected that the Europeans would have more negative $\delta^{13}\text{C}$ values than the enslaved labourers due to the likelihood that the slave diet included maize and millet, and that the enslaved labourers would have higher $\delta^{15}\text{N}$ values from consuming fish as a protein source (Varney 2003, 2011). This was because the Europeans likely did not

reside in the West Indies for long periods of time, and, while there, attempted to keep a diet similar to that of their homeland, which primarily consisted of C₃ plants and protein from terrestrial animals, while enslaved labourers were given a mix of C₃ and C₄ plants, and both terrestrial and marine protein (Buckley 1998; Dirks 1978; Duffy 1987; Dunn 1972; Sheridan 1957). Although ancestry was indeed a factor in the diet consumed by the individuals buried at the RNHC, diets were not as strictly differentiated as was expected. The European diet was consistent with expected $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, demonstrating that they were consuming foods similar to those from their homeland and had much less variability than the diets of those of African descent. It was in stable nitrogen isotope values that reflected dietary protein where the African diet differed from expected values. Stable nitrogen isotopes demonstrated that individuals of African ancestry were eating more protein from terrestrial mammals rather than marine resources. This may be because enslaved labourers owned by the Navy had a higher status than those owned by planters and received more similar foods to those of European ancestry. Thus, although both Europeans and those of African ancestry were eating protein from similar sources, their dietary staples differed, with individuals of African ancestry incorporating more C₄ foodstuffs into their diet (Varney 2003, 2011).

For a detailed methodology of stable isotope analysis used for this study, see Varney (2003). For stable isotope values used in this study see Table 5.1 in Chapter 5.

4.2.3 Investigations of Lead Burden via Trace Element Analysis

As discussed in Chapter 3, Swanston et al. (2012) used synchrotron radiation X-ray fluorescence (SR-XRF) technology to analyze the spatial distribution of lead in a sample of bone from one individual (B19a) who had been buried at the RNHC. This study concluded that lead had in fact been deposited within discrete units/osteons of the bone microstructure during life rather than in the surrounding bone tissue, which would have been indicative of postmortem contamination from the burial environment. This study has thus provided justification for the use of trace element analysis to quantify the lead in the remains of the individuals interred at the RNHC.

An initial investigation of lead burden in the bones of the RNHC population tested bone samples from 17 of the adult individuals. These samples were tested by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) in order to obtain an average lead burden in parts per million (ppm) for each individual. Lead levels obtained from this analysis resulted in a mean

bone lead level of 107.5 ppm, and a range of 13 to 336 ppm, suggesting substantial variability among the individuals interred at the cemetery. It is likely that these individuals were exposed to lead through contaminated water, contaminated rum, and potentially through medicinal compounds containing lead (Varney et al. 2015).

Further ICP-MS analysis of lead burden was carried out on the remains from the RNHC in order to test the same skeletal element (fibular diaphysis) for all of the individuals where possible. The individuals were re-tested in order to maintain consistency, given that different bones in the body take up lead at different rates due to differences in bone remodeling (Wittmers et al. 1988). Lead trace element analysis resulted in a mean bone lead level of 79.2 ppm, and a range of 10 to 251 ppm. For the trace element analysis methodology used, see section 4.3.1. Differences in bone lead levels between ancestral groups were investigated by Giffin (2014, 2015); however, no significant differences between the mean bone lead levels of those of African and those of European descent were found. Additionally, no relationship between bone lead levels and age were found for this population. It was concluded that some individuals from this population likely did suffer from lead poisoning (Giffin 2014, 2015).

4.3 Methods

4.3.1 Trace Element Analysis of Lead in Bone Samples from the RNHC

Ethics approval for sampling and analysis of the human remains from the RNHC was obtained from the University of Saskatchewan Biomedical Research Ethics Board, covered under certificate number 10-183. Samples from 24 adult and adolescent individuals interred at the RNHC were prepared for analysis via ICP-MS. Children recovered from the cemetery were not included in the samples for lead trace element analysis for several reasons: 1) the remains of the very young individuals were not complete and sampling them would have led to further destruction of their delicate bones; 2) children's bones are quite porous in comparison to older individuals and thus they are more vulnerable to the effects of diagenesis which could have a substantial effect on the lead results; 3) children absorb lead in the gastrointestinal tract, and take up lead into their bones at a different rate than adults (Agency for Toxic Substances and Disease Registry [ATSDR] 1988; Ziegler 1978), and thus would provide a poor comparative sample for this research. Samples were taken from the mid-shaft of the diaphysis of the fibula for all individuals except when this element was not available for testing. The fibula was selected in order to maximize the number of individuals for whom the same skeletal element could be

tested, as well as to select an element consisting primarily of cortical bone. Cortical bone is reflective of long-term accumulation of lead in the body, whereas trabecular bone is more variable in its lead levels and reflective of a shorter duration of lead exposure due to its higher remodeling rate (Wittmers et al. 1988). Where no fibula was available, other elements were used. For individual B8-1, a first proximal pedal phalanx was chosen; for B8-3 the right third metatarsal was chosen; and for B9a the right first metatarsal was chosen. These skeletal elements have a much larger component of trabecular bone than does the fibula. This difference in skeletal sampling may result in increased variability in skeletal lead levels for these three individuals given that the fibula is more reflective of long-term accumulation, while the pedal skeletal elements may provide lead levels representing primarily a shorter period of accumulation than cortical bone. Because lead levels in cortical bone and lead levels in trabecular bone are not directly comparable, the three individuals for whom the fibulae were not available for sampling were not included in the comparison of dietary stable isotope signatures and bone lead levels, nor in the statistical analysis of blood lead levels. However, their measurements are included in summary bone lead level and blood lead level information. Bone samples were dried and ground into a powder for trace element analysis using ICP-MS according to standard methodologies (Jenner et al. 1990; Stefanova 2003) at the Department of Geological Sciences, University of Saskatchewan. A long-term analytical error for lead measurements was determined to be $\pm 7\%$ according to quality control standard BCR-2 (Swanston et al. 2012).

4.3.2 Comparison of Dietary and Lead Data

In order to compare the stable isotope signatures from the skeletal remains of the individuals from the RNHC to their bone lead levels, the $\delta^{13}\text{C}_{\text{collagen}}$, $\delta^{13}\text{C}_{\text{apatite}}$, and $\delta^{15}\text{N}$ values were plotted in separate graphs against the lead values for easier comparison between each type of stable isotope used to reconstruct diet. First, bone lead levels were plotted against $\delta^{13}\text{C}_{\text{collagen}}$ values for the 21 individuals for whom the fibula was sampled from the RNHC in order to determine whether there was a clear pattern of association between these variables independent of the individuals' ancestries. This was done to obtain an overall impression of how lead and $\delta^{13}\text{C}_{\text{collagen}}$ values may be related to one another. Comparison of the lead and $\delta^{13}\text{C}_{\text{collagen}}$ data was done using statistical testing (outlined in section 4.3.3) and visual examination. Since sample sizes were small, the statistical analysis may not have provided the most accurate description of the relationship between the variables and it was necessary to look for associations that might not

have been detected using statistical methods that rely on the strength of linear relationships. To further analyze the variables, they were compared by ancestral group (either European or African) in the same way used to compare the variables with all individuals together. Based on the demographic composition of the rest of the cemetery and dietary reconstruction, it is possible that the category of unknown ancestry was composed of individuals of both African and European descent. Despite this, those of unknown ancestry were also analyzed separately in order to determine if they followed any particular pattern (more European, or more African) in the association between variables. Comparisons were also carried out for $\delta^{13}\text{C}_{\text{apatite}}$ and lead, as well as $\delta^{15}\text{N}$ and lead using the same methods as for $\delta^{13}\text{C}_{\text{collagen}}$ and lead.

A variety of challenges needed to be considered in the interpretation of the relationship between lead and diet. Due to the nature of the population in question, being from an archaeological context, most information pertaining to the lives of these individuals was unknown. For the individuals of European descent, it was not known how long they had served in the Navy, nor how long they had been in Antigua when they perished. This information would have given clues as to the length of time these individuals consumed naval rations and had increased exposure to rum as part of those rations. This same question applied to the individuals of African descent, who may or may not have spent the majority of their adult lives as enslaved labourers with the Navy. Moreover, exposure to lead was probably not solely through consumption of rum and contaminated sugarcane products, such as molasses (which would contribute to C_4 plant signatures in the stable isotope values), though rum may have been a substantial source of lead. Individuals may have been exposed to lead in a variety of other manners, some unrelated to diet.

4.3.3 Statistical Analysis of Diet and Lead Comparison

The objective of the statistical analysis was to compare diet and lead (through stable isotope signatures and bone lead levels, respectively) in order to determine the extent to which these variables were associated, and whether the correlation, if significant, was positive or negative. In a positive correlation, both variables increase concurrently, while in a negative correlation, one variable increases as the other one decreases. A coefficient of association is a numerical value ranging between -1.0 and 1.0. A perfect negative correlation is -1.0, while a perfect positive correlation is 1.0. These perfect coefficients of association are indicative of a perfect linear relationship between the two variables. A coefficient of 0 indicates that there is no

association between the variables. The coefficient of association is a measure of the degree to which two variables are associated, but is not indicative of a causal relationship (Champion 1971).

Testing for normal distribution of the data sets was carried out using GraphPad Software 2014. Tests used were the D'Agostino-Pearson, Shapiro-Wilke, and Kolmogorov-Smirnov tests. Due to the small sample size and because the data were not normally distributed, a non-parametric test for correlation was chosen. Spearman's rho rank-order correlation coefficient determines the magnitude of association between two variables (Champion 1971). The null hypothesis (H_0) for a Spearman's test is that there is no association between the two variables.

Spearman's correlation coefficient was calculated to determine the association between lead and each of $\delta^{13}\text{C}_{\text{collagen}}$, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}_{\text{apatite}}$ for all individuals recovered from the RNHC. A second set of calculations was carried out for the same variable pairs, but for each ancestry group separately. A significance level of $\alpha = .05$ was selected for the analysis. For the correlation between lead and each of the stable isotope signatures, the H_0 was that there is no association between lead and the isotopic values. The alternative hypothesis (H_a) was that there is an association between the two variables. The R value (r_s) indicates the strength of the association between the two variables. The p value indicates the likelihood that the correlation is the result of random sampling. The critical values for r_s for the number of individuals (n) relevant to this study are as follows: for $n = 21$, $r_s = .435$; for $n = 7$, $r_s = .786$ (Ritchey 2000).

4.3.4 Bone Lead to Blood Lead Conversions

Estimates of symptomatology associated with lead exposure could not be made directly from bone lead levels because of the lack of clinical literature relating bone lead levels to clinical lead poisoning. Clinical literature has linked blood lead levels to symptoms of lead exposure (Brodkin et al. 2007). Several archaeological studies have linked bone lead levels to estimates of symptomatology through the conversion of bone lead levels to blood lead levels (Corruccini et al. 1987; Handler et al. 1986; Keenleyside et al. 1996, 1997; Kjaer et al. 2009). One such study (Corruccini et al. 1987) converted bone lead levels to blood lead levels following the preliminary results of a study relating bone and blood lead levels in occupationally exposed workers in industrial twentieth century Britain (Somervaille et al. 1988). Although these types of conversions offer only rough estimates of actual blood lead levels at any time during an individual's life, and are in reality representative of average blood lead levels, there is currently

no other method of assessing whether or not an individual suffered symptoms of lead poisoning based on bone lead levels. It is important to note that the formula was derived for individuals with consistent exposure to lead over a known period of time and who had been monitored for blood lead levels during this period of exposure (Somervaille et al. 1988). In contrast, little is known about the archaeological population from the RNHC in terms of length of exposure and how consistent the exposure to lead was during that timeframe.

The formula utilized by Corruccini et al. (1987) was selected for this study because of the similarities in the populations studied (individuals with highly variable bone lead levels), and the ability to apply this formula to the entire lifetime of an individual given that time of exposure to lead for the individuals at the RNHC was unknown. Although the formula was derived from the association between tibia bone lead measurements and blood lead levels, Corruccini et al. (1987) used cortical bone samples from the crania and mandibles. The use of the fibula in this study is an appropriate substitute for the tibia given that the fibula, like the tibia, is primarily composed of cortical bone. For The regression formula is as follows: $\text{Blood Pb } (\mu\text{g/dL}) = \text{Tibia Pb (ppm)} / (0.03) (\text{years exposure} - 0.9)$. The formula was originally expressed as $\text{Tibia Pb } (\mu\text{g/g}) = 0.03 \times \text{Blood Pb } (\mu\text{g/100mL}) \times \text{years exposure} - 0.9$ (Corruccini et al. 1987). It was re-arranged in order to solve for the unknown variable (Blood Pb), since the bone lead levels were known. This formula was derived for use with wet bone; thus, Corruccini et al. (1987) noted that bone ash lead provides a value for blood lead at 0.531 ± 0.009 . Bone lead levels and age at death estimations (as a proxy for years of exposure) for individuals at the RNHC were used in the above formula in order to calculate blood lead levels. Because age estimates were expressed in age ranges, the average age was used in the formula (Corruccini et al. 1987).

Statistical analysis of blood lead levels is covered in section 4.3.5. Blood lead levels were presented in graphical form for visual examination. The blood lead levels calculated were then compared to clinical literature associating blood lead with symptoms of lead poisoning and the effects of lead on humans.

4.3.5 Statistical Analysis of Blood Lead Levels

Mean blood lead levels and ranges were calculated for all samples taken from the RNHC, as well as for each ancestral group separately. As previously noted, the unknown ancestry group may be composed of both individuals of European and African descent, but was analyzed in

order to determine if the individuals in this group more closely followed patterns presented by either of the aforementioned ancestral groups.

As above, the lead data were tested for normal distribution using GraphPad Software 2014, using the D'Agostino-Pearson, Shapiro-Wilke, and Kolmogorov-Smirnov tests. The non-parametric Kruskal-Wallis analysis-of-variance (ANOVA) test was chosen in order to test for a significant difference in blood lead levels between those of African descent, European descent, and unknown ancestry, due to the small sample size, and because the data were not normally distributed. The H_0 was that there is no difference in blood lead levels among those of European, African, and unknown ancestry. The H_a was that there is a difference in blood lead levels among the three groups. At a level of significance of $\alpha = .05$, an H value of ≥ 5.991 indicates that the samples (African, European, and unknown) are different from one another. An H value of < 5.991 results in a failure to reject the H_0 (Champion 1971). It should be noted that the Kruskal-Wallis test becomes less powerful with small sample sizes (GraphPad Software 2014).

4.4 Summary

The data used for this study were obtained from the skeletal remains of the individuals interred at the RNHC. These individuals were recovered from the RNHC between 1997 and 2001. Morphological analysis of the remains indicated that ages at death ranged from newborn to over 60 years of age. All adult and adolescent individuals for whom sex could be estimated were male, seven adult individuals were of African descent, one adolescent and six adult individuals were of European descent, and the remaining adult and adolescent individuals were of unknown ancestry. For this study, only adolescent and adult individuals were included.

Dietary reconstruction using stable carbon and nitrogen isotope analysis of bone collagen and stable carbon isotope analysis of bone apatite was carried out by Varney (2003). Stable isotope signatures used for this study are presented in Chapter 5, Table 5.1.

Trace element analysis was also carried out for the individuals interred at the RNHC. Trace element analysis for lead was conducted at the Department of Geological Sciences at the University of Saskatchewan. Results of the lead trace element analysis are presented in Chapter 5, Table 5.1. Analysis to determine significant differences in bone lead levels between those of African and those of European descent resulted in findings of no significant differences between the two ancestral groups (Giffin 2014, 2015).

For this study, a comparison of bone lead levels to diet was conducted through both visual examination of the graphical data as well as statistical analysis using the Spearman's rho rank-order correlation coefficient. These comparisons were done for the 21 individuals considered as a single group and separately for each ancestral group (European, African, and unknown) to determine if the correlation between diet and lead differed among the ancestral categories.

Blood lead levels were calculated following Corruccini et al. (1987) in order to estimate possible symptomatology for those individuals who may have suffered from lead poisoning. Blood lead levels were also examined visually and statistically to determine if there were significant differences between blood lead levels in those of African and those of European descent.

The results of the analysis of the relationship between the stable isotope and bone lead data are presented in Chapter 5. This chapter also includes the results of the conversion of bone lead levels to blood lead levels.

Chapter 5: Results

5.1 Introduction

A comparison of diet and lead burden was carried out for the 21 individuals exhumed from the Royal Naval Hospital Cemetery (RNHC) in Antigua for whom the fibula was sampled. This chapter presents the results of this comparison, including the statistical analysis for correlation between stable isotope signatures and bone lead levels, as well as a visual examination of the relationship between the variables. Graphs of these comparisons are provided. The results of the conversion of bone lead levels to blood lead levels are also covered in this chapter, along with statistical analysis and visual examination to determine if a significant difference exists between the blood lead levels of those of African and European descent.

5.2 Summary of Stable Isotope and Bone Lead Level Data

Table 5.1 presents the summary results of the stable isotope analysis for the individuals buried at the RNHC, including $\delta^{13}\text{C}_{\text{collagen}}$, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}_{\text{apatite}}$, carried out by Varney (2003). This table also contains the results for the quantitative bone lead (Pb) analysis in ppm, along with data on ancestry and age.

5.3 Correlation of Diet and Lead Exposure

5.3.1 Comparison of Bone Lead Levels and $\delta^{13}\text{C}_{\text{collagen}}$

Figure 5.1 summarizes the bone lead and $\delta^{13}\text{C}_{\text{collagen}}$ values in ppm and ‰ respectively for the individuals for whom the fibula was sampled for lead analysis, including those of European, African, and unknown ancestry. Using Spearman's rho rank-order correlation coefficient to determine the strength of the relationship between bone lead levels and $\delta^{13}\text{C}_{\text{collagen}}$ for the 21 individuals together resulted in an r_s value of .629 ($p = .0023$). The null hypothesis, that there is no association between these two variables, is therefore rejected at $\alpha = .05$. The alternative hypothesis, that there is a correlation between $\delta^{13}\text{C}_{\text{collagen}}$ and bone lead, is accepted. The r_s value, though statistically significant, is of moderate strength. This may be as a result of the small sample size or testing individuals of all ancestries together, as there may be a difference in strength of correlation between those of African and those of European descent.

Table 5.1 Summary Results of Stable Isotope Analysis and Bone Lead Levels

Individual	Ancestry	Age (yrs)	$\delta^{13}\text{C}_{\text{collagen}}$ (‰)	$\delta^{15}\text{N}_{\text{collagen}}$ (‰)	$\delta^{13}\text{C}_{\text{apatite}}$ (‰)	Bone Pb (ppm)
B1	A	45–49	-11.3	9.9	-6.1	41.19
B2	E	25–29	-20.4	11.9	-13.3	22.18
B3	E	30–34	-18.4	12.5	-12.2	72.25
B4	A	50–60	-10.5	12.9	-4.9	163.10
B5	E	16–18	-18.0	11.8	-10.2	90.18
B6	E	25–29	-20.8	10.8	-12.6	21.03
B8-1*	U	14–15	-19.3	11.8	-10.5	214.77
B8-3*	U	20–29	-20.1	10.7	-11.8	36.89
B9a*	U	18–20	-14.1	11.7	-8.0	149.04
B9b	U	20–29	-18.1	11.3	-8.1	151.92
B12a	A	35–39	-15.7	12.9	-6.8	86.93
B13	A	45–49	-15.5	11.3	-9.6	41.97
B14	U	35–39	-18.8	11.9	-11.6	30.90
B15a	E	35–39	-18.4	12.5	-10.8	251.49
B16	U	16–18	-18.2	12.8	-9.7	73.19
B17	U	35–39	-19.7	10.9	-12.7	15.99
B18	E	30–35	-19.3	10.4	-9.5	54.72
B19a	E	40–45	-17.0	12.2	-10.1	101.85
B19b	U	20–24	-19.0	10.2	-12.1	61.19
B22	U	20–29	-19.6	11.7	-9.0	10.08
B23	A	35–39	-19.5	11.5	-9.5	121.77
B24	A	25–29	-19.5	13.2	-10.9	42.09
B25	A	20–25	-18.9	11.3	-10.2	23.08
B26	U	14–18	-20.0	11.4	-12.5	21.70

Ancestry: A (African), E (European), U (unknown). * Indicates that these measurements were not taken from the fibula, therefore these individuals were not included in the comparison of diet to lead.

There is a visible trend shown in Figure 5.1 that, for many individuals, higher bone lead levels are coincident with more positive $\delta^{13}\text{C}_{\text{collagen}}$ values. This association is reflected in the results of the statistical analysis presented above. Individuals that follow noted trends are circled in the graph. Graphs with each data point labeled by individual can be found in Appendix I for $\delta^{13}\text{C}_{\text{collagen}}$ vs. bone lead.

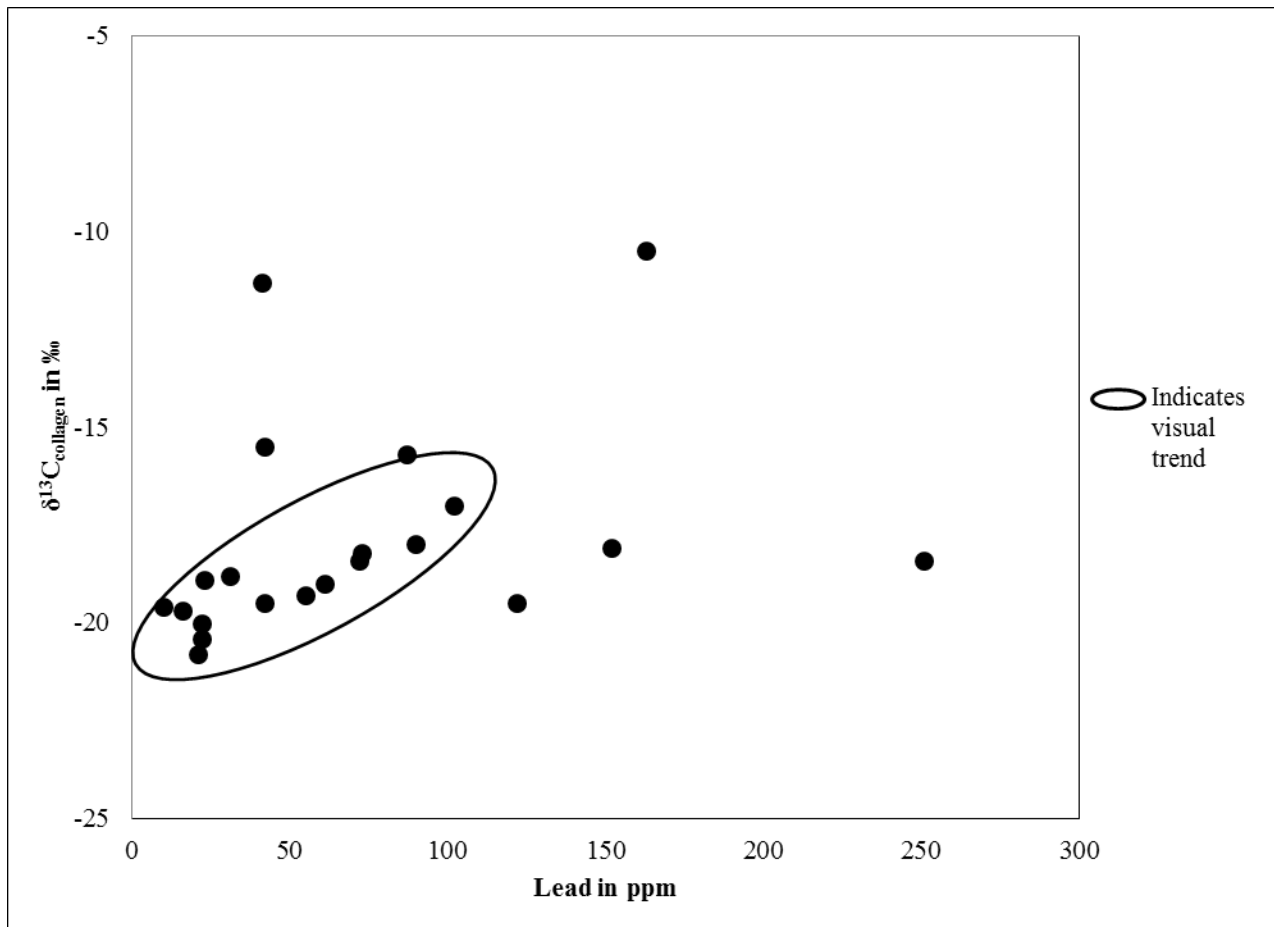


Figure 5.1 $\delta^{13}\text{C}_{\text{collagen}}$ values in ‰ vs. bone lead in ppm. Circled trends are visual, not statistically significant.

In order to further analyze the data, bone lead levels were compared to $\delta^{13}\text{C}_{\text{collagen}}$ values controlling for ancestry. This comparison is shown in Figure 5.2 for individuals of African, European, and unknown ancestry. Spearman's rho rank-order correlation coefficient was calculated for each group separately. The value of r_s for those of African ancestry is .072 ($p = .8780$). This suggests that the association between the lead levels and $\delta^{13}\text{C}_{\text{collagen}}$ is not

statistically significant, and is in fact extremely weak. The null hypothesis, that there is no association between bone lead levels and $\delta^{13}\text{C}_{\text{collagen}}$, cannot be rejected at $\alpha = .05$. For those of unknown ancestry, the r_s value is .821 ($p = .0235$), which indicates that the association between the two variables is statistically significant. For those of European ancestry, the r_s value is .847 ($p = .0162$), which indicates that the association between $\delta^{13}\text{C}_{\text{collagen}}$ and bone lead for Europeans is also statistically significant. For both those of European and unknown ancestry, the null hypothesis is rejected at $\alpha = .05$, and the alternative hypothesis, that there is an association between these two variables, is accepted. The r_s values for the latter groups are relatively strong. The importance of this relationship is discussed in Chapter 6.

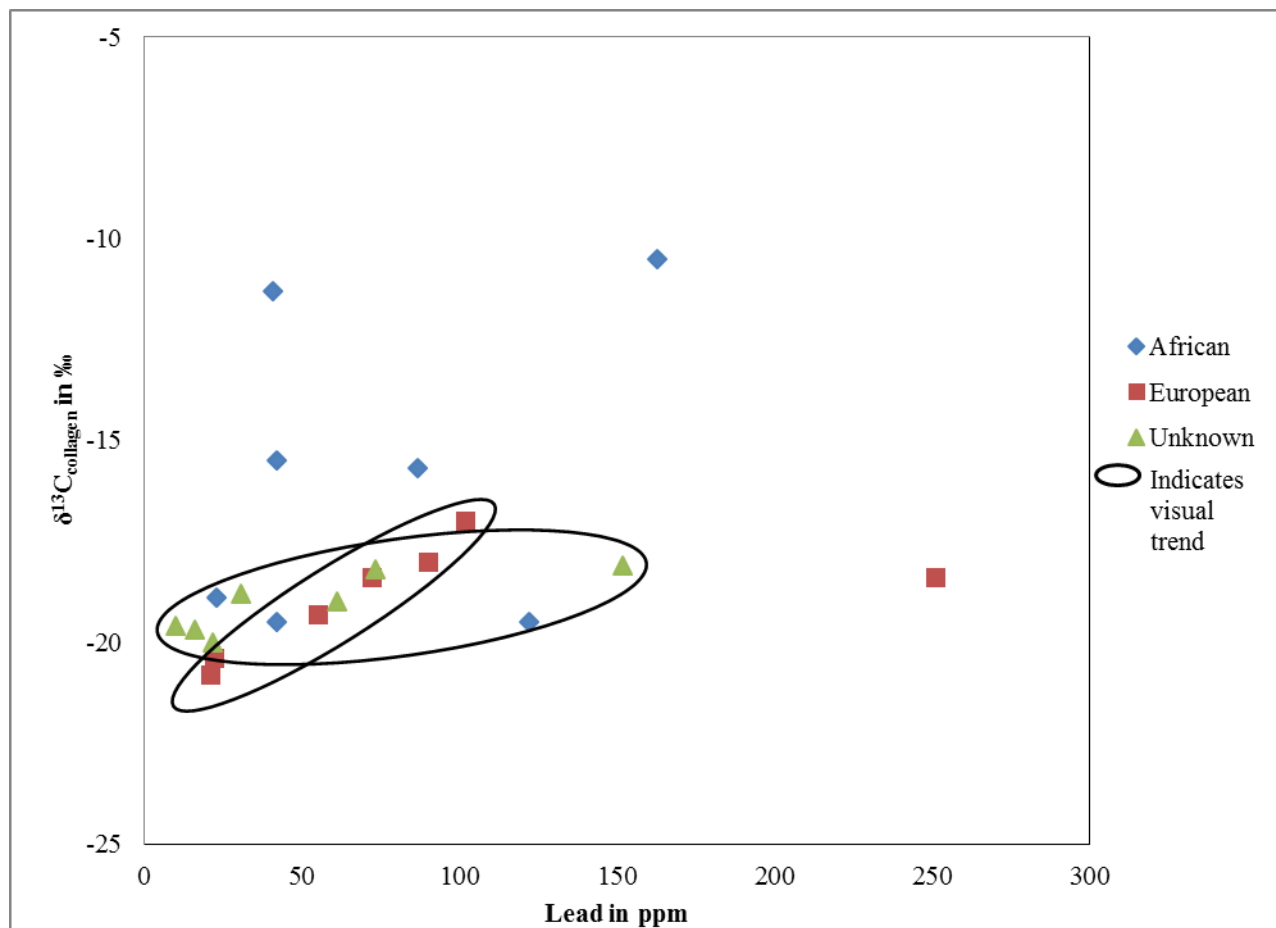


Figure 5.2 $\delta^{13}\text{C}_{\text{collagen}}$ in ‰ vs. bone lead in ppm for those of African, European, and unknown ancestries. Circled trends are visual, not statistically significant.

In Figure 5.2, there are visible trends in the presented data for both those of European ancestry and those of unknown ancestry. These trends have been circled in the graph. The most prominent trend, as reflected by the statistical analysis, is among those of European ancestry. The data demonstrate that higher bone lead levels are coincident with more positive $\delta^{13}\text{C}_{\text{collagen}}$ values, with only one outlier present. For those of unknown ancestry, the pattern of association is less linear, however, the majority of the individuals in this category do follow a similar pattern to those of European ancestry, with both variables increasing concurrently.

5.3.2 Comparison of Bone Lead Levels and $\delta^{15}\text{N}$

A summary of bone lead levels in ppm vs. $\delta^{15}\text{N}$ values from collagen in ‰ is shown in Figure 5.3 for individuals of all ancestries. To determine the strength of the correlation between $\delta^{15}\text{N}$ and lead, Spearman's rho rank-order correlation coefficient was calculated. The calculated r_s value is .424 ($p = .0556$). The null hypothesis, that there is no association between bone lead and $\delta^{15}\text{N}$, cannot be rejected at $\alpha = .05$. Though not statistically significant, the p value does approach statistical significance. However, the r_s value does not suggest a particularly strong correlation between the variables, and is in fact much weaker than the correlation between $\delta^{13}\text{C}_{\text{collagen}}$ and lead.

In Figure 5.3 there is no single clear trend in the relationship between $\delta^{15}\text{N}$ and lead. Individuals with $\delta^{15}\text{N}$ values below 11‰ do tend to have lower lead levels in their bones (< 62 ppm). Individuals with $\delta^{15}\text{N}$ values between 11‰ and 12‰ have a very large range of lead levels associated with them (10–152 ppm). Finally individuals with higher $\delta^{15}\text{N}$ (> 12‰) values have somewhat higher lead levels (> 72 ppm). This does not suggest a clear correlation between increasing $\delta^{15}\text{N}$ values and bone lead levels; however, it does suggest some difference in lead exposure based on the type and quantity of protein consumed. The noted trends are circled in the corresponding graph. Graphs with individuals labeled can be found in Appendix II for the relationship between $\delta^{15}\text{N}$ and bone lead.

Data were analyzed further by examining each ancestral group separately. Figure 5.4 shows $\delta^{15}\text{N}$ values compared with bone lead for those of European, African, and unknown ancestry. Calculation of Spearman's rho rank-order correlation coefficient resulted in r_s values of .655 ($p = .1106$) and .631 ($p = .1289$) for those of African descent and those of European descent respectively. These values result in a failure to reject the null hypothesis, that there is no association between $\delta^{15}\text{N}$ collagen and bone lead at $\alpha = .05$. For those of unknown ancestry, an r_s

value of .036 ($p = .9394$) suggests that the correlation between these variables is also not statistically significant. Thus, the null hypothesis cannot be rejected at $\alpha = .05$.

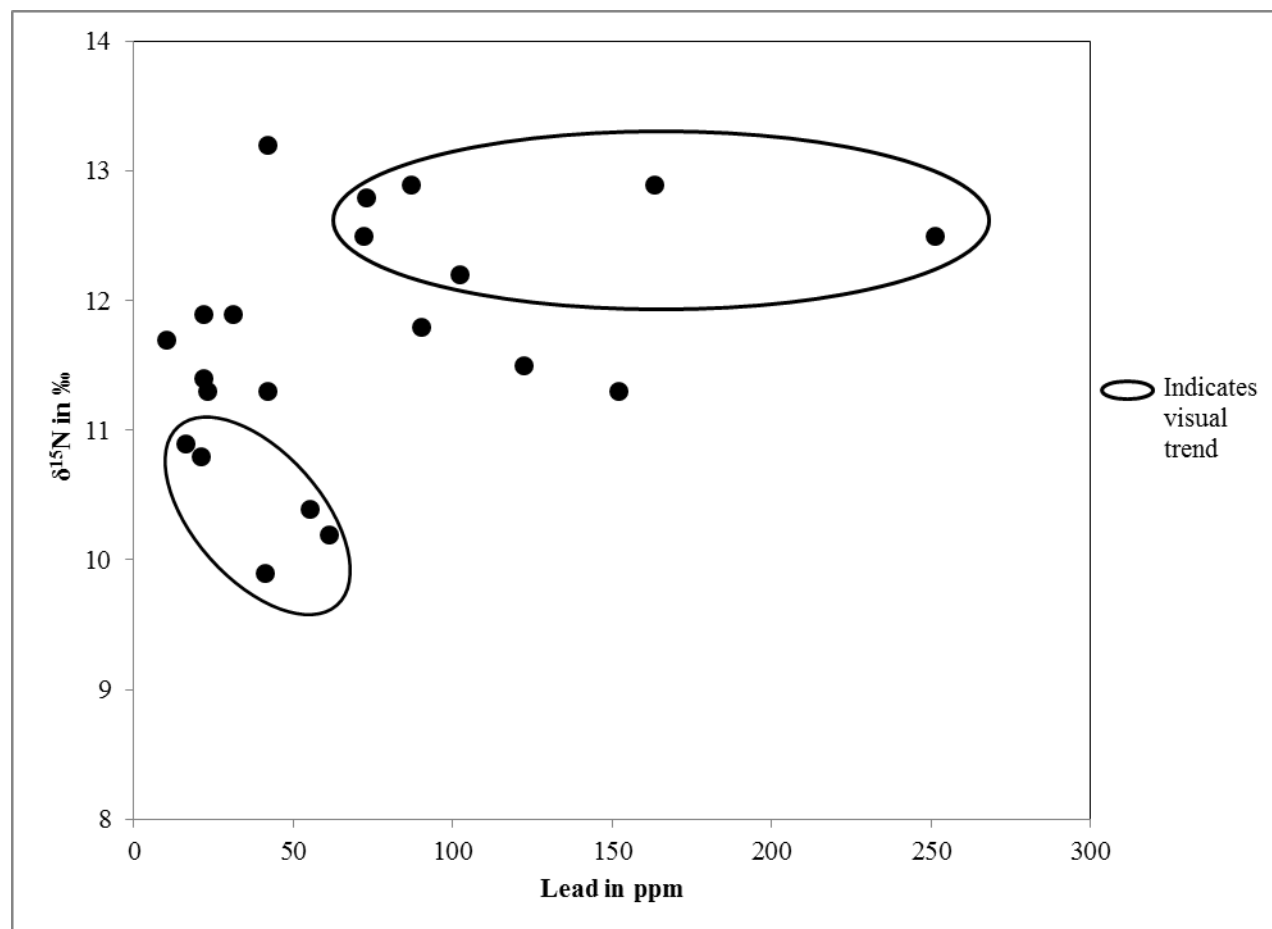


Figure 5.3 $\delta^{15}\text{N}$ in ‰ vs. bone lead in ppm. Circled trends are visual, not statistically significant.

No clear trend in the data is apparent as presented in Figure 5.4 for either of the ancestral groups, nor for those of unknown ancestry, as the lead levels and the $\delta^{15}\text{N}$ values do not increase or decrease in a similar fashion.

5.3.3 Comparison of Bone Lead Levels and $\delta^{13}\text{C}_{\text{apatite}}$

Figure 5.5 shows $\delta^{13}\text{C}_{\text{apatite}}$ in ‰ vs. bone lead levels in ppm for individuals of all ancestries combined. Spearman's rho rank-order correlation coefficient was calculated to determine the association between $\delta^{13}\text{C}_{\text{apatite}}$ and lead for all individuals. The r_s value is .474 ($p = .0301$). The correlation between these variables is considered to be statistically significant, and

thus, the null hypothesis, that there is no association between $\delta^{13}\text{C}_{\text{apatite}}$ and lead, is rejected. The alternative hypothesis is therefore accepted. Despite the association between the variables being statistically significant, the r_s value suggests that the correlation between $\delta^{13}\text{C}_{\text{apatite}}$ and lead is not particularly strong.

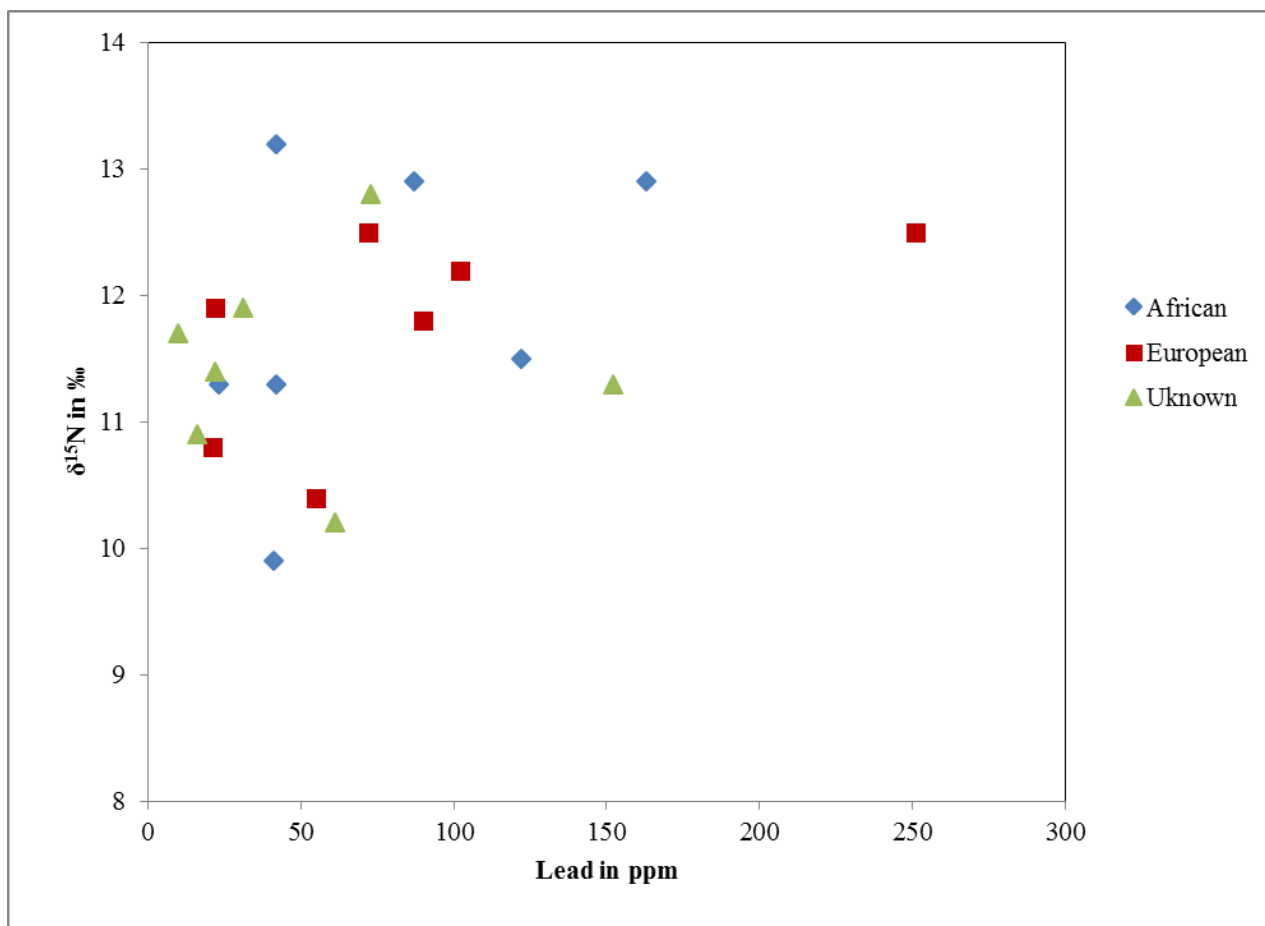


Figure 5.4 $\delta^{15}\text{N}$ in ‰ vs. bone lead in ppm for those of African, European, and unknown ancestries.

There is a slight visible trend in Figure 5.5 between $\delta^{13}\text{C}_{\text{apatite}}$ values and bone lead levels. This trend has been circled in the corresponding graph. Graphs labeled by individual for $\delta^{13}\text{C}_{\text{apatite}}$ vs. lead can be found in Appendix III. For some individuals, it appears that increasingly greater lead levels are coincident with more positive $\delta^{13}\text{C}_{\text{apatite}}$ values, although this trend is much less clear than the relationship visible between $\delta^{13}\text{C}_{\text{collagen}}$ and lead seen in Figure 5.1.

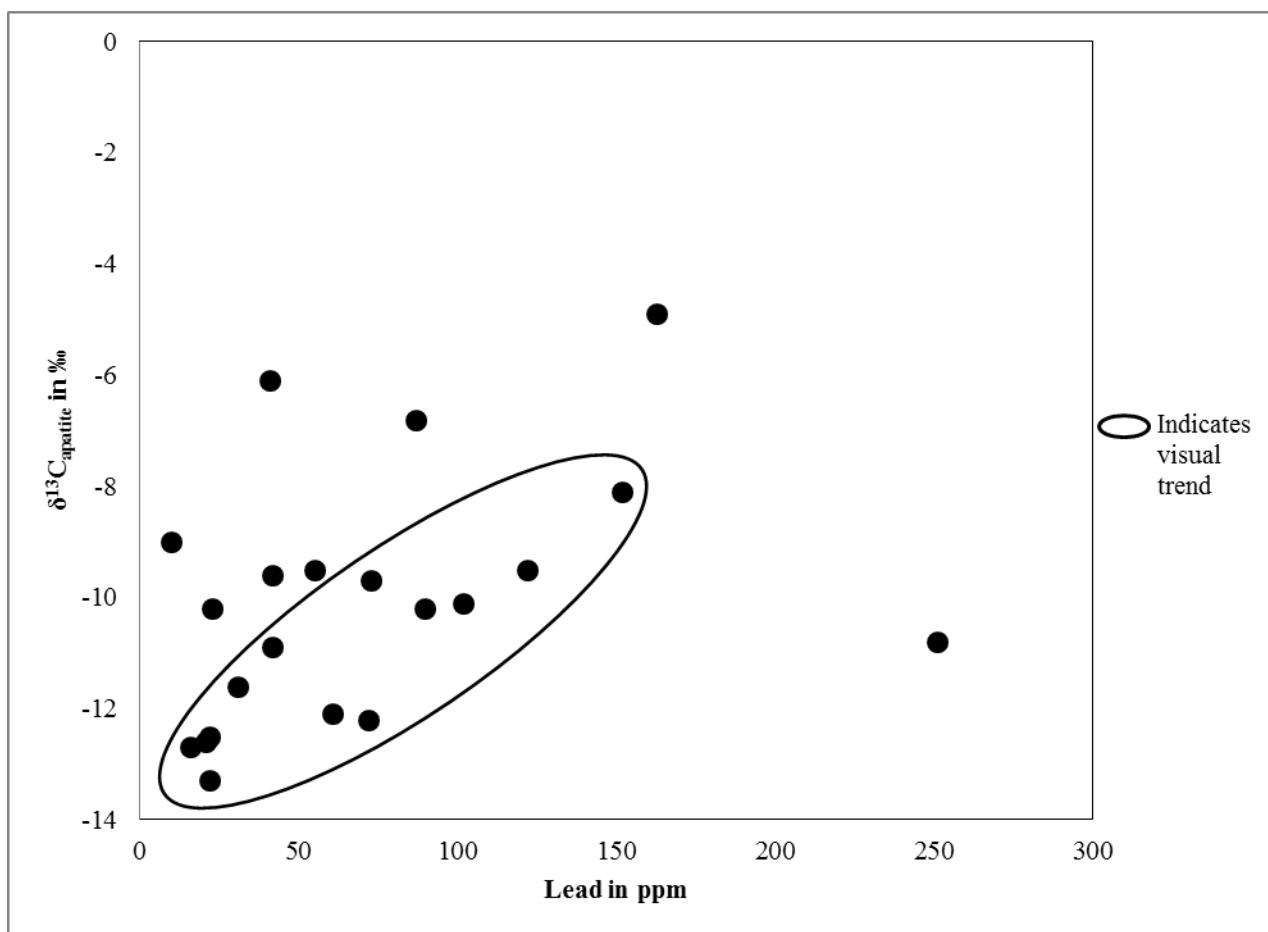


Figure 5.5 $\delta^{13}\text{C}_{\text{apatite}}$ in ‰ vs. bone lead in ppm. Circled trends are visual, not statistically significant.

Further comparisons involved separating groups by ancestry to investigate any patterns that may apply to one group but not the others. Figure 5.6 summarizes $\delta^{13}\text{C}_{\text{apatite}}$ in ‰ and bone lead in ppm for individuals of African, European, and unknown ancestry. Calculation of Spearman's rho rank-order correlation coefficient resulted in r_s values of .464 ($p = .294$) and .5 ($p = .2532$) for those of African and European descent respectively. The r_s value for Spearman's rho rank-order correlation coefficient is .429 ($p = .3373$) for those individuals of unknown ancestry. The correlation between $\delta^{13}\text{C}$ apatite and lead is not statistically significant at $\alpha = .05$ for any of the ancestral groups tested separately. The null hypothesis, that there is no association between these two variables, cannot be rejected.

There are several visible trends when the individuals are separated into groups by ancestry, though none of these is particularly strong and a trend is more evident when the

individuals are considered as one group. These trends are circled in Figure 5.6, and demonstrate that with higher lead levels, there are more positive $\delta^{13}\text{C}_{\text{apatite}}$ values. However, there are several individuals who do not follow this pattern of association. The patterns visible in the relationship between the stable isotope signatures and lead are discussed in Chapter 6.

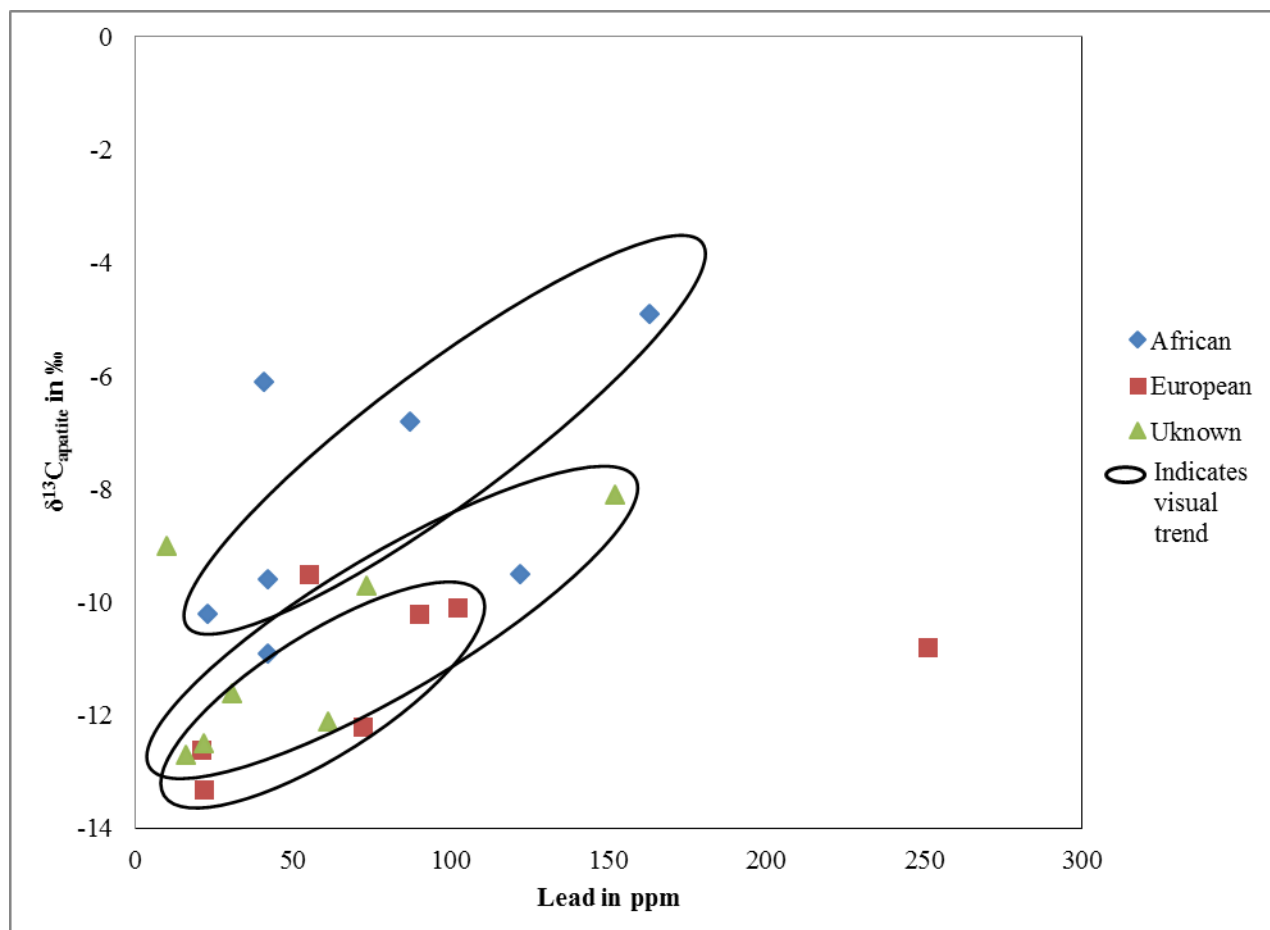


Figure 5.6 $\delta^{13}\text{C}_{\text{apatite}}$ in ‰ vs. bone lead in ppm for individuals of African, European, and unknown ancestries. Circled trends are visual, not statistically significant.

5.4 Blood Lead Levels

The formula utilized to convert bone lead levels to blood lead levels was used by Corruccini et al. (1987) following studies on occupationally exposed workers whose blood lead levels and bone lead levels were tested. The regression formula is as follows: Blood Pb ($\mu\text{g}/\text{dL}$) = Tibia Pb (ppm) / (0.03) (years exposure – 0.9). This formula was derived for use with wet

bone, thus Corruccini et al. (1987) noted that a correction factor must be used for bone ash lead by multiplying the blood lead level by 0.531 ± 0.009 . Following Corruccini et al. (1987), the average estimation of age at death for the individuals at the RNHC was used as the duration of lead exposure. Table 5.2 shows the results of the conversion to blood lead levels, along with data for age and ancestry.

The mean blood lead level for all individuals for whom the fibula was available for testing is 43.20 $\mu\text{g/dL}$. For those of African descent the mean is 33.64 $\mu\text{g/dL}$, while for those of European descent the mean is 52.74 $\mu\text{g/dL}$. Those of unknown ancestry have a mean of 43.21 $\mu\text{g/dL}$. These results may be found in Table 5.3 and Figure 5.7. Blood lead levels from all individuals range from a minimum of 7.54 $\mu\text{g/dL}$ to a maximum of 123.65 $\mu\text{g/dL}$.

In order to test for statistically significant differences among all three groups, including those of African, European, and unknown ancestry, a Kruskal-Wallis one-way analysis-of-variance was calculated. The H value at $\alpha = .05$ was .364 ($p = .8338$). The null hypothesis, that there is no difference in blood lead levels between these groups, cannot be rejected.

A visual examination of Figure 5.7 suggests, however, that individuals of European descent may have somewhat higher blood lead levels than those of African descent. All individuals of African descent have blood lead levels lower than 60 $\mu\text{g/dL}$, whereas the maximum for those of European descent is more than double that at 124 $\mu\text{g/dL}$. Individuals falling into the unknown ancestry category have a range of blood lead levels that is quite similar to those of European descent, including an individual with a blood lead level over 100 $\mu\text{g/dL}$, and the lowest blood lead level for the sample tested at 8 $\mu\text{g/dL}$. Although the statistical analysis suggests that there is no statistically significant difference in blood lead levels between any of these groups, a visual examination demonstrates that there is some difference at higher blood lead levels, and that those falling into the category of African ancestry have lower blood lead levels on the whole than those of European descent. This difference is discussed in more detail in Chapter 6.

Table 5.2 Blood Lead Levels Calculated Using the Average Age at Death Estimation as Duration of Lead Exposure

Individual	Ancestry	Age (yrs)	Bone Pb	Blood Pb
B1	A	45–49	41.19	15.75
B2	E	25–29	22.18	15.10
B3	E	30–34	72.25	41.25
B4	A	50–60	163.10	53.46
B5	E	16–18	90.18	99.76
B6	E	25–29	21.03	14.32
B8-1*	U	14–15	214.77	282.04
B8-3*	U	20–29	36.89	27.67
B9a*	U	18–20	149.04	146.56
B9b	U	20–29	151.92	113.94
B12a	A	35–39	86.93	42.74
B13	A	45–49	41.97	16.15
B14	U	35–39	30.90	15.15
B15a	E	35–39	251.49	123.65
B16	U	16–18	73.19	80.97
B17	U	35–39	15.99	7.86
B18	E	30–35	54.72	31.14
B19a	E	40–45	101.85	43.97
B19b	U	20–24	61.19	51.57
B22	U	20–29	10.08	7.54
B23	A	35–39	121.77	59.87
B24	A	25–29	42.09	28.65
B25	A	20–25	23.08	18.85
B26	U	14–18	21.70	25.44

Ancestry: A (African), E (European), U (unknown); Bone Pb in ppm; Blood Pb in $\mu\text{g/dL}$. * Indicates individuals for whom the fibula was not available for testing, and were therefore not used in the statistical analysis of blood lead levels.

Table 5.3 Mean and Range Blood Lead Levels in $\mu\text{g/dL}$

Ancestry	Mean blood lead	Minimum blood	Maximum blood
African	33.64	15.75	59.87
European	52.74	14.32	123.65
Unknown	43.21	7.54	113.94
All	43.20	7.54	123.65

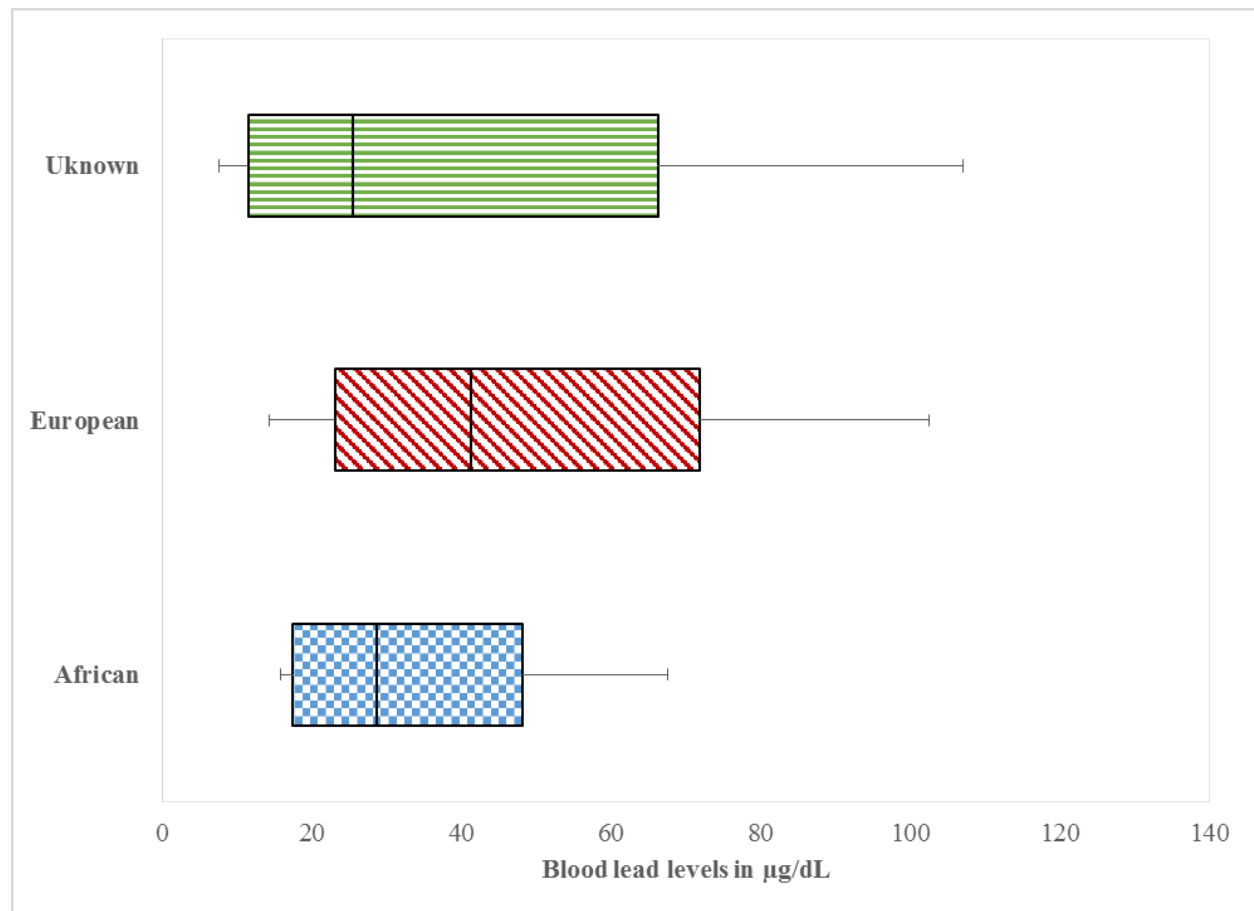


Figure 5.7 Blood lead levels in $\mu\text{g/dL}$ according to ancestry.

5.5 Summary

The objectives of this chapter were to compare stable isotope data to bone lead data for individuals buried at the RNHC in order to discern any relationship between diet and lead exposure, and to estimate blood lead levels from bone lead levels.

Stable isotopes, including $\delta^{13}\text{C}_{\text{collagen}}$, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}_{\text{apatite}}$ were each compared to bone lead for all ancestries combined and subsequently for each group, African, European, and unknown, separately. For $\delta^{13}\text{C}_{\text{collagen}}$ vs. bone lead for all individuals combined, the correlation between the two variables was considered to be statistically significant; however, the strength of the relationship was not particularly strong. A visual examination suggested that, for many of the individuals, more positive $\delta^{13}\text{C}_{\text{collagen}}$ were coincident with increasingly higher bone lead levels. When the variables were compared by separating each group by ancestry, for those of African descent, the correlation between $\delta^{13}\text{C}_{\text{collagen}}$ and lead was not statistically significant. In contrast, the association between $\delta^{13}\text{C}_{\text{collagen}}$ and lead for those of European and unknown ancestry was statistically significant, and relatively clear patterns were demonstrated in the graph for the relationship between these two variables.

Stable nitrogen isotopes were also compared to lead to determine if there was a strong correlation between these variables. Statistical analysis of the correlation between $\delta^{15}\text{N}$ and lead for all ancestries combined resulted in an association between the variables that was not statistically significant. A visual examination suggested that there was no clear pattern of association between these variables. With each group analyzed separately, the strength of the correlation for those of European and African descent was extremely similar, but neither was considered to be statistically significant. For those of unknown ancestry, the correlation between $\delta^{15}\text{N}$ and lead was much weaker and also not statistically significant.

Stable carbon isotopes obtained from bone apatite were compared to bone lead for all ancestral groups combined. Similar to $\delta^{13}\text{C}_{\text{collagen}}$, the correlation between $\delta^{13}\text{C}_{\text{apatite}}$ and bone lead was considered to be statistically significant, although the relationship was weaker than that of $\delta^{13}\text{C}_{\text{collagen}}$ and lead. The distribution of the data points in the comparative chart suggested a similar relationship between $\delta^{13}\text{C}_{\text{apatite}}$ and lead, and $\delta^{13}\text{C}_{\text{collagen}}$ and lead. Again, the relationship was much weaker for $\delta^{13}\text{C}_{\text{apatite}}$ and lead than for $\delta^{13}\text{C}_{\text{collagen}}$ and lead. When each group was analyzed separately by ancestry, the strength of the correlation between $\delta^{13}\text{C}_{\text{apatite}}$ and lead for those of African, European, and unknown descent was very similar, but not statistically

significant for any of these groups. For all three groups there was a visual trend that suggested that, for some of the individuals, more positive $\delta^{13}\text{C}_{\text{apatite}}$ values were coincident with higher lead levels; however this relationship was not strong for any of the groups.

Blood lead levels for the individuals at the RNHC were calculated following the work of Corruccini et al. (1987). The range of blood lead levels was from a minimum of 7.54 $\mu\text{g/dL}$ to a maximum of 123.65 $\mu\text{g/dL}$, indicating a large variation in lead exposure. The mean blood lead level for all individuals was 43.20 $\mu\text{g/dL}$. There was no statistically significant difference in blood lead levels between those of African and those of European descent. A visual examination of the data suggested, however, that those individuals falling into the European and unknown ancestry categories had, in general, somewhat higher blood lead levels than those of African descent. The limitations of converting bone lead levels to blood lead levels through regression formulae are discussed in Chapter 6.

The results presented in this chapter, including the relationship between diet and lead, and the estimated blood lead levels are interpreted in Chapter 6.

Chapter 6: Discussion

6.1 Introduction

This chapter discusses the results presented in Chapter 5. Interpretations focus on the relationship between diet, as reconstructed using stable isotope analysis, and bone lead levels. Additionally, this chapter examines estimated symptomatology based on blood lead levels, as calculated from bone lead levels. Challenges to data interpretation are also examined.

6.2 Correlation between Diet and Lead

In the interest of clarity, the relationship between diet and lead is initially discussed as separate comparisons between each stable isotope signature ($\delta^{13}\text{C}_{\text{collagen}}$, $\delta^{15}\text{N}$, $\delta^{13}\text{C}_{\text{apatite}}$) and lead. Then, all the comparisons between stable isotope signatures and bone lead levels are discussed together in order to establish a more comprehensive interpretation of the association between diet and lead.

6.2.1 Comparison of $\delta^{13}\text{C}_{\text{collagen}}$ and Bone Lead Levels

From the results presented in Chapter 5, and visible in Figure 5.1, the relationship between $\delta^{13}\text{C}_{\text{collagen}}$ and lead, for the 21 individuals analyzed regardless of ancestry, demonstrates that many individuals fall into a pattern of more positive $\delta^{13}\text{C}_{\text{collagen}}$ values coinciding with increasingly higher bone lead levels for distinct individuals. However, this is not true for all individuals and, when the data are separated into ancestral groups (as seen in Figure 5.2), it becomes apparent that those individuals of European and unknown ancestry tend to fit the aforementioned pattern far better than those in the African ancestry category. Although sample sizes are quite small, which increases the possibility that observed patterns in the data may be misleading as a result of chance, the tendency for those of unknown ancestry to follow a similar pattern to those of European ancestry suggests that many of these individuals may have been of European rather than of African descent.

A direct relationship between $\delta^{13}\text{C}_{\text{collagen}}$ and lead would likely be the result of consumption of substantial quantities of lead-contaminated food products, such as rum or other contaminated C_4 items that would be distinguishable in the stable isotope signatures. An indirect relationship would be the result of an association between a particular dietary regime and higher or lower bone lead levels; however, the source of the lead exposure would not be distinguishable

in the stable isotope signatures. Given that $\delta^{13}\text{C}_{\text{collagen}}$ tends to be biased towards the protein component of diet (Ambrose and Norr 1993) it is more likely that the relationship observed between $\delta^{13}\text{C}_{\text{collagen}}$ and lead in Figure 5.2 is as a result of an indirect relationship between foods consumed and bone lead levels, but it is not impossible that the energy component of diet could have affected the $\delta^{13}\text{C}_{\text{collagen}}$ values for many of the individuals studied. The relationship between dietary protein and lead can be further examined in the association with $\delta^{15}\text{N}$, and is discussed below.

There are three possible explanations for the association between $\delta^{13}\text{C}_{\text{collagen}}$ and bone lead seen in Figure 5.2, but they are not necessarily mutually exclusive:

First, the results are due to chance. This seems unlikely due to the strong correlations between the variables for Europeans and those of unknown ancestry, and a very weak correlation for those of African ancestry. These results suggest that there is a real difference between the association of $\delta^{13}\text{C}_{\text{collagen}}$ and lead that is affected by ancestry.

Second, the results are due to a direct relationship between $\delta^{13}\text{C}_{\text{collagen}}$ and bone lead. Although $\delta^{13}\text{C}_{\text{collagen}}$ is biased towards the protein component of diet, a direct relationship may be observable if the quantity of protein in the diets consumed was insufficient to completely mask the contribution of other dietary components. The energy component of diet does contribute to the stable isotope signatures in bone collagen, but at a considerably reduced capacity in comparison to protein (Ambrose and Norr 1993). Ambrose and Norr (1993) suggested that, in a diet of 20% protein, energy sources, such as carbohydrates, would contribute 29–34% of the carbon in collagen. Though rum contains no carbohydrates in the form of sugars, the alcohol may still have had an impact on stable carbon isotope signatures in bone. Thus, the consumption of rum and other lead-contaminated sugarcane products, though free of protein, may still have a small, but potentially distinguishable, effect on the $\delta^{13}\text{C}$ values of bone collagen.

Third, the results are due to an indirect relationship between $\delta^{13}\text{C}_{\text{collagen}}$ and lead. Instead of lead-contaminated foodstuffs having a direct effect on the stable isotope signatures of bone collagen, individuals eating a certain type of diet may have been exposed to more or less lead. Although the protein component of the naval diet primarily consisted of imported salted meats (Blane 1785; Buckley 1998; Dyde 1997), fresh local meat was occasionally available from the Codrington plantation on Barbuda (Higman 1984; Nicholson 2002) or from local markets. Animals in Europe would most likely have consumed a diet based on C_3 grains, while those

animals in the West Indies likely ate a mixed diet of C₃ and C₄ plants (Klippel 2001; Varney 2003). The pattern observed for many individuals in the relationship between $\delta^{13}\text{C}_{\text{collagen}}$ and bone lead could demonstrate that individuals who were consuming more local meats were also exposed to more lead, potentially through increased access to locally produced lead-contaminated rum. Although the meat itself was likely not contaminated with lead, this relationship may be seen in two ways. First, because fresh meat was a more desirable and costly commodity, individuals of higher status may have had increased access to it along with other desirable food items. Second, independent of status, individuals who consumed more locally raised animals and had higher lead levels may have spent more time in the West Indies than those individuals with lower lead levels and more negative $\delta^{13}\text{C}_{\text{collagen}}$ values. Individuals who spent more time in the Caribbean and more time on land would almost certainly have had more access to both increased quantities of local meat and also increased quantities of lead-contaminated rum, given that, on the ships, the rations of alcohol were strictly controlled and salted meats were primarily consumed by lower-ranking personnel (Blane 1785; Pack 1982).

Since $\delta^{13}\text{C}$ values from bone collagen are biased towards the protein component of diet, it is probable that the third explanation presented above is the most likely and that the association observed between $\delta^{13}\text{C}_{\text{collagen}}$ and bone lead levels reflects an indirect relationship between the consumption of locally raised animals and increased access to lead-contaminated goods, which is probably due to a longer duration of stay in the West Indies. Although this provides a viable explanation for the overall relationship seen between $\delta^{13}\text{C}_{\text{collagen}}$ and bone lead, it does not explain the substantial differences identified for those of African and European descent in the correlation between the variables.

As can be seen in Figure 5.2, individuals of African ancestry do not follow any detectable pattern in the relationship between $\delta^{13}\text{C}_{\text{collagen}}$ and lead. The variability seen for those of African ancestry is in stark contrast to the linear pattern demonstrated for those of European descent and many individuals of unknown ancestry. There are two possibilities for the weak relationship between the variables for those of African ancestry:

First, those of African descent in the West Indies were known to take their rationed food to market to obtain goods that they preferred (Buckley 1979; Dyde 1997; Luffman 1789). Moreover, in comparison to those of European ancestry, they probably spent more time, or even their entire lives in the West Indies. Thus, they ate a more varied diet that, for some of the

individuals of African descent, contained far more C₄ foodstuffs and potentially more C₄ fed animals than the diet consumed by the Europeans, which was based more on C₃ staples. Therefore, it is possible that, due to the consumption of additional and larger quantities of C₄ foods by some of those of African descent, the same pattern of association between lead exposure and diet may not hold true for individuals of different ancestry. For those of European ancestry, consuming a diet based primarily on C₃ staples and C₃ fed terrestrial animals, consumption of limited quantities of C₄ fed animals is discernible in the stable isotope signatures. In contrast, due to the quite variable diet consumed by those of African ancestry, including C₃ and C₄ staples, and C₃ and C₄ fed terrestrial animals, changes in diet that are coincident with bone lead levels are not observable to the same degree. It is noteworthy that three individuals of African ancestry (B23, B24 and B25) fall very close to the patterns of association between $\delta^{13}\text{C}_{\text{collagen}}$ and lead exhibited by those of European and unknown ancestry. Probably these individuals consumed primarily naval rations rather than locally available C₄ foods.

Second, although individuals of African descent owned by the Navy received rations of rum, Buckley (1979:105) suggested that they were not “addicted” to it in the same way as the European naval personnel. In fact, this was one of the reasons why the West Indian regiments composed of African and Afro-Caribbean individuals were more effective and preferred over regiments composed of European individuals (Buckley 1979). Even if rum did not have a considerable impact on $\delta^{13}\text{C}_{\text{collagen}}$ values, it was still thought to be a major contributor to lead exposure at the time, and likely had a substantial influence on bone lead levels. If individuals of African descent interred at the RNHC were consuming much less rum than Europeans, or more varied quantities of rum based on personal preference or social influences, then a clear pattern of correlation between $\delta^{13}\text{C}_{\text{collagen}}$ and lead is unlikely to be seen for these individuals.

Based on these two explanations for the difference in the strength of correlation between the variables for those of European and African ancestry, it is possible, and indeed likely, that both the consumption of more varied foodstuffs and the less consistent consumption of rum contributed to the lack of a correlation between $\delta^{13}\text{C}_{\text{collagen}}$ and lead levels for those of African descent.

From the results of the comparison of $\delta^{13}\text{C}_{\text{collagen}}$ and bone lead, it can be suggested that, for the majority of individuals consuming a diet consisting primarily of C₃ staple foods and

terrestrial animals fed with C₃ plants, there is a relatively strong correlation between diet and bone lead, likely as a result of the indirect relationship between the consumption of more locally-raised animals and increased access to lead-contaminated foods such as rum. In contrast, for individuals likely eating a mixed diet with C₄ and C₃ plants, and C₄ fed terrestrial animals, there is not a strong correlation between diet and bone lead levels. It cannot be discounted that there may have been alternative sources of exposure to lead, such as occupational exposure or exposure through non-comestible goods, which is discussed in section 6.2.5. It is evident that ancestry plays a central role in dietary differences among the individuals interred at the RNHC, which has also had a considerable effect on the existence of a strong correlation between $\delta^{13}\text{C}_{\text{collagen}}$ and lead.

6.2.2 Comparison of $\delta^{15}\text{N}$ and Bone Lead Levels

Based on the results presented in Chapter 5, there is no direct relationship between bone lead and $\delta^{15}\text{N}$ in the sense that consumption of lead-contaminated foods would have a discernible impact on $\delta^{15}\text{N}$ stable isotope signatures. This is expected given that the $\delta^{15}\text{N}$ signatures from bone collagen are more indicative of consumption of foods at the higher end of the trophic level spectrum, foods with high concentrations of nitrogen, and foods higher in protein (DeNiro and Epstein 1981; Makarewicz and Sealy 2015; Phillips and Koch 2002). Not only is rum free of protein but it also has negligible quantities of nitrogen (Delavante 2004), making it a non-contributor to $\delta^{15}\text{N}$ values. Thus, any relationship inferred from the data must be an indirect relationship in which bone lead levels are associated with consumption of certain types or quantities of protein, but not as a result of these foods being contaminated with lead themselves.

There is some grouping of the $\delta^{15}\text{N}$ data that seems to be independent of ancestry. Those individuals with lower $\delta^{15}\text{N}$ values (<11‰) tend to have lower bone lead levels than those with higher $\delta^{15}\text{N}$ values (> 12‰). However, with one individual (B24, $\delta^{15}\text{N}$ = 13.2‰, lead = 42.09 ppm), there is some overlap, given that this individual has a relatively low bone lead level and the highest $\delta^{15}\text{N}$ value for this population. Those individuals with $\delta^{15}\text{N}$ values between 11‰ and 12‰ have variable bone lead levels ranging from very low to very high (10–152 ppm). This suggests that the apparent association between low bone lead levels and low $\delta^{15}\text{N}$ values, and high bone lead levels and high $\delta^{15}\text{N}$ values, may actually be the result of sample bias due to the small number of individuals recovered from the RNHC and used in this study. However, if the distribution of data presented in Figures 5.3 and 5.4 is an accurate portrayal of the relationship

between $\delta^{15}\text{N}$ and bone lead, the association between the variables may be as a result of the type of animal protein that was consumed. High $\delta^{15}\text{N}$ values may be associated with the consumption of locally raised animals, indicating that individuals consuming more fresh, local meat had higher bone lead levels than individuals consuming more imported meats. Animals raised in the West Indies may have had higher $\delta^{15}\text{N}$ values than animals raised in Europe for two reasons. The practice of manuring fields, done in some parts of the West Indies during the colonial period (Sheridan 1985), results in an enrichment of $\delta^{15}\text{N}$ values of plants grown in this environment (Styring et al. 2014). This enrichment is then passed on to animals feeding on these plants (Varney 2003). An additional reason for more positive $\delta^{15}\text{N}$ values for animals raised in the West Indies is the effect of water stress on the $\delta^{15}\text{N}$ signatures of plants and animals in areas where drought is frequent. In arid environments, $\delta^{15}\text{N}$ values tend to be more positive than expected (Ambrose 1986, 1991; Schoeninger and DeNiro 1984; Schwarcz et al. 1999; Sealy et al. 1987). Antigua, and particularly Barbuda, are quite dry and subject to periods of drought, and in some cases water had to be imported (Berleant-Schiller et al. 1995; Sheridan 1985). Thus, $\delta^{15}\text{N}$ values of imported meats may have been lower than the $\delta^{15}\text{N}$ values of meat from animals raised on Antigua and Barbuda (Varney 2003). Although consumption of more imported salted meats vs. local fresh meat may be correlated with lower and higher lead levels respectively for those at the highest and lowest ends of the $\delta^{15}\text{N}$ spectrum, there is a great deal of variability for individuals with $\delta^{15}\text{N}$ values between 11‰ and 12‰.

Values for $\delta^{15}\text{N}$ are not only affected by the type of protein consumed, but also by the quantity. Individuals with higher $\delta^{15}\text{N}$ values may have been consuming larger quantities of animal protein than those individuals with lower $\delta^{15}\text{N}$ values. Differences in $\delta^{15}\text{N}$ values have been used in studies of gender and status differences, with more positive $\delta^{15}\text{N}$ values indicative of consumption of larger quantities of animal protein (Ambrose et al. 2003; Le Huray and Schutkowski 2005). Thus, the variability in the association between $\delta^{15}\text{N}$ values and bone lead levels may have been not only the result of consumption of local vs. imported meats, but also affected by the quantity of meat consumed by each individual. The consumption of large quantities of salt provisions (beef and pork) provided by the Navy was considered to go hand-in-hand with increased consumption of rum to quench the thirst caused by the intake of excess salt (Dyde 1997). Higher $\delta^{15}\text{N}$ values may also be indicative of the duration of service to the Navy, as servicemen tended to have a more protein-rich diet than working class civilians (Roberts et al.

2012). However, not all of the variability observed in the association between $\delta^{15}\text{N}$ values and bone lead can be explained by the consumption of local meats and higher quantities of animal protein. This suggests that a more complex relationship exists between the variables.

A third alternative for the association between higher bone lead levels and higher $\delta^{15}\text{N}$ values results from the effects that both disease and nutritional stress may have on $\delta^{15}\text{N}$ values and bone lead levels. Given that the individuals recovered from the RNHC were probably quite ill prior to death, which resulted in their stay at the hospital, it is certainly possible that extended periods of illness could have affected lead uptake and retention of the heavier nitrogen isotope. Many diseases affect the rate of turnover in bone (Gryn timer 1993), and thus may lead to increased deposition of lead in the bone and release of lead from bone into the blood. Vitamin and mineral deficiencies may also have an effect on lead uptake in the body (Millar et al. 2015; Cheng et al. 1998), which will be discussed further in section 6.3.1. Illness and nutritional stress not only have the capacity to affect lead incorporation into bone, they also have the potential to affect $\delta^{15}\text{N}$ signatures, resulting in more positive $\delta^{15}\text{N}$ values (Katzenberg 2000; Katzenberg and Lovell 1999). From the RNHC sample, only two individuals had visible pathological conditions on their skeletal remains. Individual B17 had cribra orbitalia and individual B25 had porotic hyperostosis, which are two potentially related skeletal pathological conditions for which the etiology has been debated (Walker et al. 2009). Walker et al. (2009) suggest that porotic hyperostosis is linked to megaloblastic anemia, rather than iron-deficiency anemia, caused by a vitamin B₁₂ deficiency. They also suggest that cribra orbitalia is caused by a codeficiency of vitamin C and B₁₂. Although both these individuals likely suffered from nutritional stress at some point in their lives given that the conditions were not in their active stages at the time of death, neither exhibits excessively elevated bone lead levels or particularly high $\delta^{15}\text{N}$ values. However, research on avian tissues has demonstrated enrichment of $\delta^{15}\text{N}$ values in birds that are fasting or nutritionally stressed, suggesting that insufficient protein in the diet does affect the stable nitrogen isotope signatures in bone collagen (Hobson et al. 1993). For the individuals interred at the RNHC it seems unlikely that they would not have received sufficient dietary protein. Although increased $\delta^{15}\text{N}$ values have also been found in pathological bone in comparison to normal bone in the same individual as a result of recycling of tissue proteins due to a wasting condition, it is only bone deposited during illness that would be affected (Katzenberg and Lovell 1999). Thus, a long-term illness would have been necessary to have an

impact on the $\delta^{15}\text{N}$ values of the individuals interred at the RNHC. Though it is possible that health status may have affected the $\delta^{15}\text{N}$ values for the individuals interred at the RNHC, there was no clear indication that the $\delta^{15}\text{N}$ values were altered due to any long-lasting health condition, time being essential to have had an effect on stable isotope signatures in bone. Therefore, this explanation for the relationship between $\delta^{15}\text{N}$ values and bone lead levels is plausible, but given the lack of evidence for higher $\delta^{15}\text{N}$ values due to extended illness, it does not seem the most probable explanation for the observed relationship.

Although ancestry appears not to play a substantial role in affecting the strength of the relationship between $\delta^{15}\text{N}$ values and bone lead levels, it may indeed have indirect implications for the interpretation of this relationship. For those individuals of European ancestry, a connection between consumption of local meats and/or consumption of higher quantities of salted provisions and high bone lead levels may shed light on the duration of their stay in the West Indies and how much time they were permitted on shore. Access to rum was more controlled on board ships, but personnel on land had more opportunity to trade with locals for this much desired, but also contaminated, beverage (Pack 1982). Those Europeans staying longer in the West Indies most likely would have obtained more lead-contaminated rum and probably consumed more local meat than individuals who stayed a relatively short time. Since the relationship between the variables is not particularly consistent, other variables may have affected both the $\delta^{15}\text{N}$ values and the bone lead levels. In contrast, although those individuals of African descent were provided with naval rations, they may or may not have eaten them consistently, some individuals preferring to sell their rations to obtain more desirable foods. This type of activity was not, however, restricted to enslaved labourers, as some Europeans also chose to sell their rations to obtain a greater variety of foods (Buckley 1998). For those of African descent, status or desire to emulate those of higher status, may have played a role in the provision of certain foods through rations or the decision to obtain these foods by other means. Fresh meat was typically more expensive than preserved meats (Dunn 1972) and thus may have been more desirable for many individuals. If emulation of those of European descent was an objective of some individuals of African descent as a result of the hierarchy of the British Naval system and society in general, this may have had an effect on whether or not they chose to consume the rations of rum provided to them, thus potentially increasing their intake of lead. The moderate correlation between $\delta^{15}\text{N}$ and bone lead for those of African descent demonstrates that

agency likely played a considerable role in the extent of the exposure to lead for these individuals. For those of European descent, a similar moderate correlation between the variables would likely be both as a result of duration of time spent in the West Indies and in service of the Navy, but also as a result of agency, as some individuals may not have been as keen to consume large quantities of rum despite the social pressures of being in the Navy.

6.2.3 Comparison of $\delta^{13}\text{C}_{\text{apatite}}$ and Bone Lead Levels

As can be seen in the results from Chapter 5, there is a great deal of variability in the relationship between $\delta^{13}\text{C}_{\text{apatite}}$ and lead. This is demonstrated by the relatively weak correlation between the variables. The weakness of the correlation is unexpected, as it seemed probable that the strongest correlation between stable isotope signatures and bone lead levels would be observed between $\delta^{13}\text{C}_{\text{apatite}}$ and lead. This was based on the assumption that a direct relationship might be visible between the consumption of rum, an energy component and C_4 product, and bone lead levels, given that $\delta^{13}\text{C}_{\text{apatite}}$ is reflective of the whole diet as opposed to influenced primarily by the protein component of diet (Ambrose and Norr 1993). Some individuals, representing both ancestral groups do follow a trend of more positive $\delta^{13}\text{C}_{\text{apatite}}$ coinciding with higher bone lead levels. However, the pattern is not consistent across individuals. The data are most informative when considered by ancestral group, even though the group of unknown ancestry is probably composed of individuals of both African and European ancestry. This is due to the fact that there is a substantial difference in $\delta^{13}\text{C}_{\text{apatite}}$ signatures between blacks and whites, demonstrating that in general, those of African descent consumed more C_4 staple foods than those of European descent (Varney 2003).

For those of European ancestry, four individuals do conform to a trend of more positive $\delta^{13}\text{C}_{\text{apatite}}$ values coincident with higher bone lead levels. Three individuals do not; however, it can be presumed that one individual, B15a, is an outlier, given his extremely high bone lead levels relative to the majority of the individuals interred at the RNHC. Additionally, it cannot be expected that individuals would conform to a perfectly linear pattern given the many factors that could affect both stable isotope signatures and exposure to lead. Thus, it is, in reality, only individual B18 who deviates substantially from the pattern hypothesized. It appears that he consumed a greater quantity of C_4 staple foods, many of which were likely not contaminated with substantial quantities of lead and had little effect on his bone lead levels. On the other hand, his bone lead levels (54.72 ppm) are high enough that it is not inconceivable that he was

consuming some lead-contaminated products. Thus, although other C₄ foods were likely consumed by those of European ancestry, contaminated rum may also have contributed to the $\delta^{13}\text{C}_{\text{apatite}}$ values of these individuals.

Individuals of unknown ancestry seem to follow a similar trend to that of the individuals of European ancestry, however at somewhat more positive $\delta^{13}\text{C}_{\text{apatite}}$ values, as demonstrated in Figure 5.6. Five of seven individuals of unknown ancestry conform quite well to the aforementioned trend. It cannot be assumed that the individuals who follow this pattern are all of one or the other ancestry. The data point belonging to individual B19b falls more closely to the pattern formed by those of European descent than the pattern formed by those of unknown ancestry. In contrast, individual B22 has the lowest bone lead levels recorded for the RNHC, yet evidently consumed some C₄ staples as part of his diet. The pattern of association between the variables shown in Figure 5.6 for those of unknown ancestry suggests that, in general, individuals consuming more C₄ foods had somewhat higher bone lead levels.

Four individuals of African ancestry also follow a pattern of more positive $\delta^{13}\text{C}_{\text{apatite}}$ values coinciding with higher bone lead levels, but three do not fall into the same pattern as the other individuals of African ancestry. For those of African ancestry, the association between diet and lead is most complex given the variability shown in the foods they were consuming. Some individuals ate considerably more C₄ foods than others, and it is highly unlikely that even a large quantity of rum would have had such a dramatic effect on $\delta^{13}\text{C}_{\text{apatite}}$ values. Thus, these individuals were eating more foods such as maize as their principal carbohydrate. In contrast, other individuals consumed far less in terms of C₄ foods and have a diet more similar to those of European ancestry. Although, as previously noted, some individuals do conform to a pattern of more positive $\delta^{13}\text{C}_{\text{apatite}}$ values coincident with higher bone lead levels, the number of individuals is so small that this may be as a result of chance rather than a clear association between the variables. This may also be the case for those of European and those of unknown ancestry as well, given the very small sample sizes once individuals have been separated by ancestry.

The results of the analysis of the association between $\delta^{13}\text{C}_{\text{apatite}}$ and lead suggest that, in some cases, it may be accurate to state that the consumption of contaminated foodstuffs has a direct and discernible effect on the stable isotope signatures. However, this does not hold true for all individuals from the RNHC. It can be proposed, therefore, that variables other than the consumption of certain quantities of contaminated resources have a substantial impact on the

stable isotope and bone lead data for the individuals tested. These variables will be considered in section 6.2.5.

6.2.4 Comparison of All Dietary Signatures and Bone Lead Levels

It would be remiss not to consider the relationship between diet and lead within the context of all the stable isotope data. This approach provides a more holistic picture of the association between the stable isotope signatures and bone lead levels. It is surprising that the strongest correlation between diet and lead is found in the relationship between $\delta^{13}\text{C}_{\text{collagen}}$ and lead rather than the hypothesized $\delta^{13}\text{C}_{\text{apatite}}$ and lead (the role of ancestry will be discussed below). This demonstrates that the consumption of rum is not the primary indicator of higher bone lead levels when compared with stable isotope signatures. It does suggest, however, that for many of the individuals interred at the RNHC, the consumption of C_4 protein (likely from C_4 fed animals) is a stronger indicator of bone lead levels than the consumption of C_4 energy components. Given that C_4 fed animals were most likely local to the West Indies and that animals with higher $\delta^{15}\text{N}$ values were also most likely local to the West Indies, the finding that both more positive $\delta^{13}\text{C}_{\text{collagen}}$ and more positive $\delta^{15}\text{N}$ values are more strongly correlated with higher bone lead levels is consistent. However, when considered at the individual level, those individuals with high $\delta^{15}\text{N}$ values do not always have $\delta^{13}\text{C}_{\text{collagen}}$ values that demonstrate the consumption of local meats, and rather show that they were consuming more C_3 protein. This suggests that, although in general this pattern tends to be true for many individuals, other factors, such as larger quantities of animal protein, or even health status may have played a role in the $\delta^{15}\text{N}$ values. Although the correlation between $\delta^{13}\text{C}_{\text{apatite}}$ and bone lead levels is not strong, the trends observed in Figures 5.5 and 5.6 are not inconsistent with the consumption of rum having an effect on both the stable isotope signatures and the bone lead levels. The variability seen in these graphs is likely due to both the variety of other C_4 foods available for consumption in the West Indies and to the diversity of factors that affect bone lead levels, which will be discussed in section 6.2.5. These findings also demonstrate that the relationship between diet and lead is complex given that the consumption of certain foods is influenced greatly by both personal choice and social structures, and it is likely that at least some of the lead exposure that occurred during life for these individuals was the result of consuming contaminated food products.

Ancestry certainly played a considerable role in the relationship between diet and lead. It appears that the variability observed in the diets consumed by those of African descent,

particularly in the $\delta^{13}\text{C}$ values of both bone collagen and apatite, had an impact on the ability to see clear trends in the association between foods consumed and bone lead levels. In general, those of African descent were consuming more variable amounts of C_4 foods than those of European descent, which seems to have affected the complexity of the relationship between the variables. Those of European descent, who were consuming more C_3 staples than those of African ancestry, seem to have a more visible and simple relationship between the variables. It is also of interest that the relationship between $\delta^{15}\text{N}$ and lead appears not to have been strongly influenced by ancestry as seen in Figure 5.4, in contrast to the association between $\delta^{13}\text{C}$ and lead. This is likely because there was no statistically significant difference between the $\delta^{15}\text{N}$ values of foods consumed between those of African and those of European ancestry (Varney 2003).

Thus, based on the above interpretations, it can be established that the relationship between diet and lead is most strongly correlated for those individuals consuming a more C_3 based diet (protein and energy) than for those consuming a more varied diet including substantial quantities of C_4 foods. In the case of the RNHC, this is primarily descriptive of the diet consumed by those of European ancestry who had access to foods and beverages from the West Indies during a more restricted period of time compared to those who may have lived in the West Indies for most of their lives. It appears then that those individuals of European descent consuming more foods found in the West Indies, rather than Europe, are most likely to have higher bone lead levels. This is not the case, however, for those of African descent, for whom the association between diet and lead is more complex. In such a situation, other avenues of lead exposure unrelated to diet, such as occupational exposure, should be considered.

6.2.5 Assumptions and Challenges in Interpreting the Relationship between Diet and Lead

A variety of assumptions were made based on historical information in order to interpret the relationship between diet and lead. One of these assumptions was that the majority of the lead in the remains of the individuals buried at the RNHC had its origins in contaminated rum and other contaminated sugarcane products such as molasses and refined sugar. Additionally, it was assumed that consumption of rum, a C_4 plant product, would be discernible from the stable isotope signatures, allowing for a direct connection to be made between contaminated foodstuffs and bone lead levels. This was only expected to be possible for carbon stable isotope signatures and not for nitrogen stable isotope signatures for the reasons already presented in this chapter. Another assumption made is that the individuals of African descent

buried at the RNHC were most likely King's Negroes owned by the Navy for the majority of their lives, resulting in these individuals being of higher status than plantation labourers and likely receiving naval rations.

Although these assumptions may have been justifiable due to historical and archaeological evidence, they also mask a variety of alternative ways in which individuals interred at the RNHC may have been exposed to lead. First, although historical evidence suggests that rum was one of the primary contributors to lead poisoning for European members of the Navy (Buckley 1978), there were other sources of lead to which both European and African individuals may have been exposed. Lead was found in many places during the colonial period, including water catchment equipment, which would have resulted in contaminated drinking water, serving and table wares, such as lead glazed ceramics and utensils made with lead, and lead-based medicinal products (Eisinger 1982; McCord 1953b; Nicholson 1995; Wedeen 1984). All individuals may have been exposed to varying degrees to these sources of lead which would not have had an effect on stable carbon isotope signatures. Another source of lead exposure restricted to plantation enslaved labourers would have been exposure during the processing of sugarcane or rum distillation (Handler et al. 1986), which would be a possibility if a plantation labourer had been rented to the Navy or purchased by the Navy, after having worked on a plantation, and then died at the Naval hospital. These are some of the ways in which the individuals at the RNHC may have been exposed to lead without having any effect on their dietary signatures whatsoever.

Other confounding factors that could not be, or only partially, taken into consideration during analysis of the data were the duration and extent of lead exposure for the individuals studied, what effect age had on the lead levels, and the health status of the individuals in question. With respect to the duration and extent of lead exposure, due to the tendency for lead to accumulate in bone, an individual exposed to large quantities of lead for several years might have similar bone lead levels to an individual exposed to small quantities of lead over their entire lifetime. Thus, a younger adult individual with a high bone lead level was likely exposed to larger quantities of lead than an older individual with a similar bone lead level. Although this applies to adult individuals, adolescent individuals have a higher bone remodeling rate than adults (International Commission on Radiological Protection [ICRP] 1995). Two of the three adolescent individuals included in the analysis and interpretation of the relationship between diet

and lead did have relatively high bone lead levels, though they were well within the range exhibited by the adult individuals. With regards to age, although removing the adolescent individuals from the analysis may have eliminated some uncertainty with regards to the variability of lead uptake into the bones, the adolescents fit well within the patterns of association between diet and lead established for adults, and removing them would have been detrimental to the sample size. The inability to separate the individuals by age, due to the already small sample sizes, and the unknown duration of exposure to lead may have skewed the interpretation of the comparison of bone lead and stable isotope data. Additionally, as previously noted, bone remodeling can be affected by some diseases (Grynpas 1993), which, in cases of rapid bone turnover could increase the amount of lead incorporated into bone, as well as that released from bone into the blood. Thus, though the dose of lead might be steady, if an individual were suffering from an illness that affected his rate of bone turnover, the quantity of lead incorporated into the bone might increase. Though it can be assumed that the individuals interred at the RNHC were ill prior to death, there was limited available information on health and nutritional status, duration and nature of illness, and duration and extent of lead exposure, making it difficult to assess the effect any of these factors may have had on both the bone lead levels (Grynpas 1993; Millar et al. 2015; Somervaille et al. 1988), and stable isotope signatures, which may also be affected by disease (Katzenberg and Lovell 1999).

Another variable that may have had an effect on bone lead levels is diagenesis. Although the study by Swanston et al. (2012) indicates that at least some of the lead contained in the bones of the individuals interred at the RNHC had a biogenic origin, there is still the prospect that some lead in the remains of these individuals had a diagenetic origin and was not incorporated into the bone during life. As previously noted, it is not possible to separate biogenic from diagenetic lead in trace element analyses.

The most difficult challenge to overcome in the interpretation of the relationship between diet and lead is the small sample size available for this study. Although the total number of individuals analyzed from the RNHC ($n = 21$) has allowed for statistically significant results of correlation analysis to be obtained, when the individuals are divided into groups by ancestry, the samples become extremely small and more difficult to test statistically. Even for the visual examination of the data, such small sample sizes lead to questions of whether or not the

distribution of the data points is the result of chance or a true relationship between diet and lead that could be found in other similar populations, if tested.

6.3 Blood Lead Levels

6.3.1 Blood Lead Levels and Symptoms of Clinical Lead Poisoning

Clinical studies have rarely attempted to determine the relationship between bone lead levels and symptomatology due to the simplicity with which blood may be tested for lead exposure in living patients. Thus, in clinical practice, blood lead levels are most commonly used to determine whether an individual has been exposed to unacceptable levels of lead (Brodkin et al. 2007; Handler et al. 1986). In order to assess the veracity of the conjecture that lead poisoning may have been a major contributor to the downfall of the British military in the West Indies as suggested by Buckley (1978), blood lead levels for the individuals buried at the RNHC were calculated from the bone lead levels according to the formula used by Corruccini et al. (1987), presented in Chapters 4 and 5. From these blood lead levels, it is possible to estimate whether the individuals suffered symptoms of lead poisoning in life. Although individual variation and the actual dosage of lead may play a considerable role in how symptoms are expressed, blood lead levels have been associated with typical symptoms experienced by individuals falling within particular ranges of lead in the blood (Hernberg 1980).

Lead exposure has an effect on a variety of body systems, including the hematopoietic system, the peripheral nervous system (PNS), the central nervous system (CNS), the gastrointestinal system, the hepatic system, the reproductive system of both males and females, the cardiovascular system, and the renal system (Flora et al. 2012; Health Canada 2011; Hernberg 1980). Despite causing a wide array of dysfunctions in an organism, in cases of chronic exposure, lead poisoning (or West India dry gripes) is typically described historically as causing syndromes of the gastrointestinal, neuromuscular, and neurological systems (Cadwalader 1745; Handler et al. 1986; Hunter 1796; Pearce 2007). Handler et al. (1986) follow this model by categorizing organ systems affected by ranges of blood lead levels, including the intestinal tract, nerves, and brain. For the purposes of this research, the focus will be on symptoms affecting the gastrointestinal, and peripheral and central nervous systems, as these systems were noted to be affected in historical accounts (Cadwalader 1745; Handler et al. 1986; Hunter 1796). Gastrointestinal symptoms include abdominal pain, constipation, nausea, vomiting, indigestion, loss of appetite, and weight loss (Hernberg 1980). Presentation of a combination of these

symptoms is often referred to as colic (ATSDR 2007). Neuromuscular symptoms include a slowing of motor conduction velocity of nerves of the upper limb (Damstra 1977), and peripheral nerve impairment (Health Canada 2011). Neurological symptoms include muscular tremor, headache, loss of memory, irritability, poor concentration, hallucinations, delirium, convulsions, and coma. Presentation of a combination of these symptoms is referred to as encephalopathy, referring to diseases of the brain, and, when severe, can result in death (ATSDR 2007). For a more encompassing description of all known effects of lead on the human body the “Toxicological Profile for Lead” compiled by the Agency for Toxic Substances and Disease Registry (ATSDR) may be referenced.

In any discussion of symptomatology related to blood lead levels, it must be noted that there is not an exact consensus among researchers on what levels of lead in the blood will result in visible symptoms. In the past it has been proposed that symptoms affecting the gastrointestinal, PNS, and CNS are not manifested until blood lead levels of 80 µg/dL have been reached (Awad El Karim et al. 1986; Damstra 1977). In reality, symptoms are quite variable in terms of association with blood lead levels and subjects have reported symptoms at much lower blood lead levels (Awad El Karim et al. 1986; Baker et al. 1979; Rosenman et al. 2003). Flora et al. (2012) suggest that levels between 40–60 µg/dL resulting from chronic lead toxicity could cause severe symptoms including encephalopathy, and coma. Chronic lead toxicity may also result in a variety of health effects, including hypertension, high blood pressure (Health Canada 2011) and nephrotoxicity (Ekong et al. 2006), at lower blood lead levels (<10 µg/dL). Pearce (2007) states that unsafe levels of lead begin at 10 µg/dL, while Flora et al. (2012) suggest that there is no safe level of lead exposure. In modern clinical practice, chelation therapy to treat lead poisoning is typically employed when blood lead levels reach 45 µg/dL (Pearce 2007), suggesting that even lower levels of lead exposure are of concern. Modern treatment for levels above 80 µg/dL may be only partially successful in the prevention of permanent damage to body systems (Damstra 1977). Table 6.1 associates blood lead levels with possible symptoms based on a variety of studies of subjects exposed to lead occupationally or accidentally, and on previously compiled data related to archaeological studies of lead (ATSDR 2007; Awad El Karim et al. 1986; Baker et al. 1979; Handler et al 1986; Hernberg 1980; Rosenman et al. 2003).

It must also be noted that inter-individual variation also plays a role in the expression of negative health effects due to lead exposure (Mahaffey 1977; Millar et al. 2015). A key factor in

the manifestation of symptoms of lead poisoning is the nutritional status of the individual (Millar et al. 2015). Both vitamin and mineral deficiencies may affect lead levels in the blood and bones; vitamin C and iron deficiencies have been associated with higher lead levels in the blood, and low dietary intake of vitamin D have been associated with higher lead levels in bones (Cheng et al. 1998). Researchers have also proposed that genes affect an individual's susceptibility to lead. At least three genes may influence lead bioaccumulation and toxicokinetics, including genes that are involved in heme synthesis, vitamin D reception, and iron transport (Onalaja and Claudio 2000). Additional factors that may affect the toxicity of lead for different individuals include stress, preexisting disease, and advanced age (Kosnett et al. 2007; Millar et al. 2015).

Table 6.1 Blood Lead Level Ranges and Associated Symptoms*

Symptoms	0-30µg/dL	30-60µg/dL	60-80µg/dL	80-100µg/dL	100-200µg/dL	200 + µg/dL
Gastrointestinal	Unlikely	Possible Loss of appetite Abdominal pain Mild colic	Probable Mild colic	Probable Colic	Probable Severe colic	Probable Severe colic
Neuromuscular	Unlikely	Possible Extensor muscle weakness Pain or soreness in muscles Nerve conduction impairment	Possible Extensor muscle weakness Joint and muscle pain Nerve conduction impairment	Probable Impaired movement of limbs	Probable Impaired movement of limbs	Probable Paralysis of extensor muscles
Central Nervous System	Unlikely	Possible Irritability Headaches Poor concentration Fatigue	Possible Irritability Headaches Poor concentration Fatigue	Possible Encephalopathy	Probable Encephalopathy	Probable Severe encephalopathy Possible Coma

* Data from ATSDR (2007); Awad El Karim et al. (1986); Baker et al. (1979); Handler et al (1986); Hernberg (1980); Rosenman et al. (2003)

For blood lead levels ranging between 0–30 µg/dL, it is unlikely that individuals would notice any symptoms of lead exposure and would be classified as asymptomatic. Of the 21 RNHC individuals for whom blood lead levels were calculated from the fibula bone lead measurement, 10 individuals (or 48%) would likely have been considered asymptomatic, having blood lead levels below 30 µg/dL. Of those that fall within the asymptomatic category, four are of African descent, two of European descent, and four are of unknown ancestry. Individuals with blood lead levels ranging from 30–60 µg/dL may be asymptomatic; however, there is a possibility that lead exposure at these levels may affect the gastrointestinal tract, peripheral nerves and muscles, as well as the CNS, resulting in mild colic, muscle weakness, irritability, and headaches. Seven individuals (or 33%) from the RNHC fall within this category. Three of the individuals are of African descent and three are of European descent, and one individual is of unknown ancestry. None of the individuals from the RNHC falls within the blood lead level range of 60–80 µg/dL. Blood lead levels between 80–100 µg/dL are likely to result in symptoms of colic, impaired movement of the limbs, and symptoms of encephalopathy. Two individuals (or 9.5%) from the RNHC may have had blood lead levels within this range. One of the individuals is of European descent, and one is of unknown ancestry. Blood lead levels between 100–200 µg/dL are likely to affect the intestinal tract, the PNS, and the CNS. Individuals may suffer from vomiting, constipation, nausea, weight loss, anorexia, impaired movement of the limbs, and various symptoms of encephalopathy. Two individuals (or 9.5%) from the RNHC have estimated blood lead levels within this range. Of these, one individual is of European descent and one individual is of unknown ancestry. Finally, those individuals with over 200 µg/dL blood lead levels are likely to be severely impaired by lead poisoning. These levels would result in intestinal tract colic with muscle spasms, paralysis of the extensor muscles, and the potential for coma which could be life threatening. There were no individuals with blood lead levels over 200 µg/dL. Figure 6.1 provides a summary of the number of individuals by ancestry that fall within each range of blood lead levels.

The calculated blood lead levels are based on average levels for the entire lifetime of the individuals in question, rather than the actual period during which these individuals were exposed to lead because the duration of exposure is unknown for all of the individuals at the RNHC. Thus, it is probable that these blood lead level estimates underestimate the true blood lead levels that these individuals may have exhibited for at least some portion of their lives.

Based on the above discussion of lead poisoning symptoms at given blood lead levels, it is possible that approximately 52% of the individuals from the RNHC expressed some degree of clinical symptoms of lead poisoning. Thirty-three percent of the total number of individuals likely had milder symptoms, while 46% were probably asymptomatic. Only 20% of the individuals from the RNHC were likely to have experienced more severe symptoms of lead poisoning.

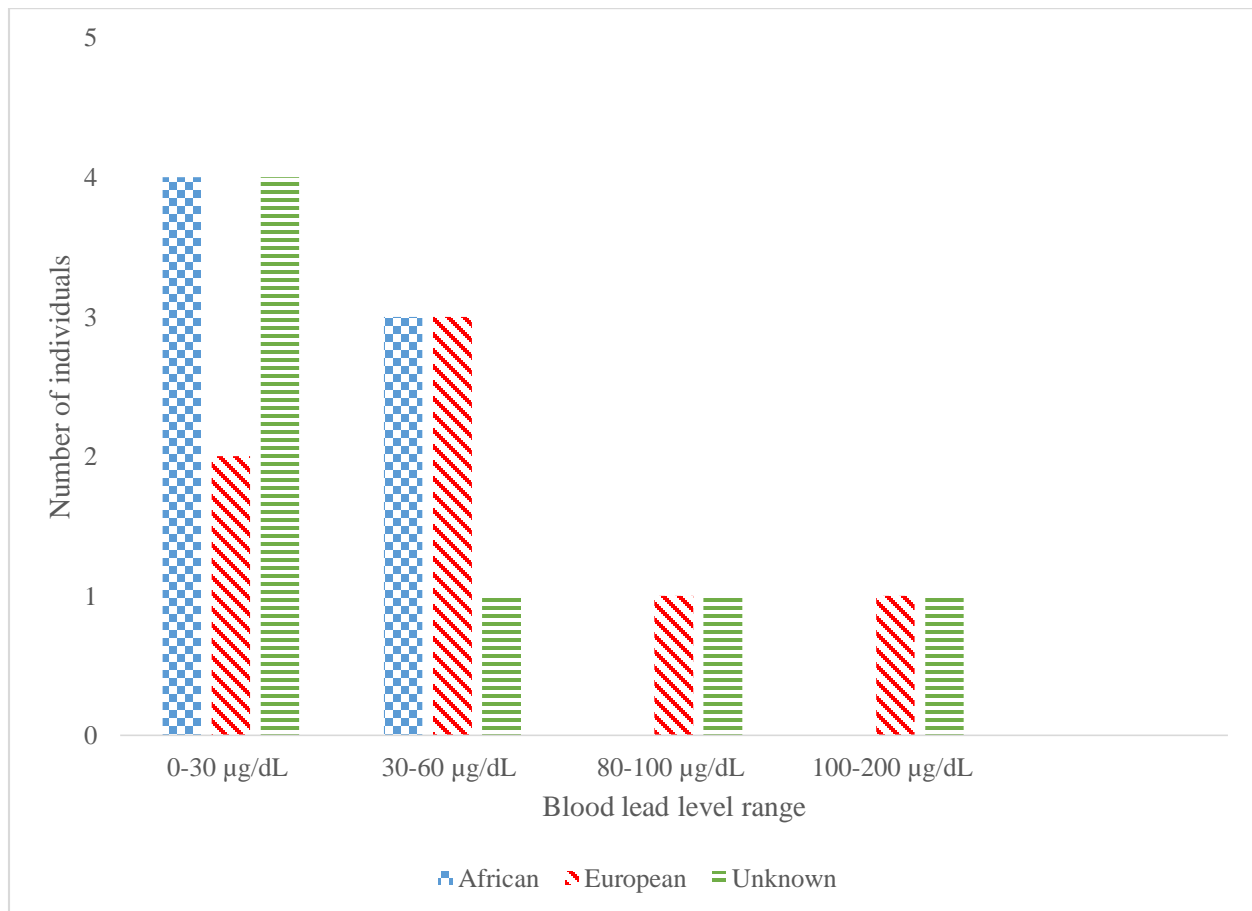


Figure 6.1 Summary of individuals falling within each blood lead level range.

Although the majority of the individuals interred at the RNHC undoubtedly suffered few or no symptoms of lead poisoning during their lifetime, the results of this study are not inconsistent with the assertion that lead poisoning had a considerable impact on the health and well-being of the individuals serving in the British military in the West Indies. In fact, the synergistic relationship of various health concerns, including poor nutrition, alcoholism, lead

poisoning, infectious disease, and the ill effects of a tropical climate for those unaccustomed to it, would have served to compound the symptoms of any illness suffered by those serving in the British military. Social phenomena, such as poverty, may have also exacerbated the effects of ill health (Singer et al. 2011). The effects of lead exposure were probably far more devastating to individuals considered to be of lower status and whose health was already precarious than for otherwise healthy and high status individuals (Buckley 1978; Duffy 1987; Dunn 1972; Singer et al. 2011). Consequently, although the blood lead level estimations provide a guide through which the extent of the effects of lead as a toxin may be understood within the context of the colonial West Indies, there may well have been as-yet unknown factors which also could have played a role in the expression of symptoms of lead poisoning in the individuals from the RNHC.

6.3.2 Differences in Blood Lead Levels Based on Ancestry

Based on the statistical results presented in Chapter 5, there is no difference in blood lead levels among any of the three ancestral categories tested. However, it is clear from visual examination that several individuals of European and unknown ancestry had somewhat higher blood lead levels than those of African ancestry. This is reflected in the assessment of the extent to which individuals may have experienced symptoms of lead poisoning, with individuals of African descent having blood lead levels that likely would have resulted in less severe symptoms. In contrast, a small number of individuals of European ancestry may have suffered more severe symptoms of lead poisoning. Although it would appear that those of African descent were less likely to suffer from symptoms of lead poisoning, many individuals of European descent also had quite low blood lead levels. Because of this variability in blood lead levels, it cannot be supposed that the individuals interred at the RNHC had differential access to contaminated goods or differential occupational exposure based on ancestry, though either were certainly possibilities. It is conceivable, however, that other factors, such as agency or social influences, played a considerable role in the extent to which individuals were exposed to lead, based on the desirability and utilization of particular goods. Historical sources have noted that those of African descent were not as disposed to drinking large quantities of rum as those of European descent (Buckley 1979). It must be noted though, that the amount of time spent in the West Indies for all of these individuals may also have had a substantial effect on exposure to lead.

6.3.3 Discussion of the Challenges Associated with Blood Lead Level Estimations

As stated above, the conversion of bone lead levels to blood lead levels represents a rough estimate of the average blood lead levels of an individual during exposure to lead and thus, also a rough estimate of associated symptomatology. A variety of factors, including the incorporation of lead into soft and skeletal tissues, bone turnover, acute vs. chronic lead exposure, and individual variation may affect the accuracy of these conversions, particularly in the case of an historic population about which individual details are unknown. Lead travels via the blood and is deposited in soft tissues and bone, but the length of time it remains in each of these compartments varies greatly. The mean half-life of lead in blood is approximately 36 days, while lead can remain in the skeleton for years to decades (Brodkin et al. 2007; Rabinowitz et al. 1976). Bone lead levels are considered to be representative of long-term lead exposure (Aufderheide 1989), or in other words the total body burden of lead (Brodkin et al. 2007). Blood lead levels typically give an indication of recent lead exposure rather than cumulative exposure (Brodkin et al. 2007; Graziano 1994; Somervaille et al. 1988), as demonstrated in Figure 6.2. To further complicate the issue of blood lead levels, Graziano (1994) noted that investigators generally accept a second half-life for blood lead levels that is approximately 4 years due to the release of lead from the bone back into the blood. This lead may then be excreted or redeposited back into bone (Aufderheide and Wittmers 1992). It is evident that blood and bone lead levels represent two distinct measurements of lead in body tissues, though they are related and affect one another over time. Brodkin et al. (2007) suggested that blood lead levels are a poor indicator of the accumulation of lead in bone. Ahlgren et al. (1980) determined that there is no simple relationship between lead levels in the blood and lead levels in the skeleton.

The research conducted by Somervaille et al. (1988), which was adapted by Corruccini et al. (1987) to estimate blood lead levels for a population of enslaved labourers on Barbados, was done to establish the relationship between blood lead levels and bone lead levels in occupationally exposed workers from the UK in the twentieth century. In contrast to this modern population, the individuals at the RNHC may have been exposed to lead through a variety of ways including occupational exposure, but also likely through the consumption of lead-contaminated foodstuffs. The twentieth century-workers in the UK were monitored to ensure that their blood lead levels never exceeded a maximum of 70 µg/dL (Somervaille et al. 1988), whereas the exposure to lead for the individuals at the RNHC went completely unchecked and

for some individuals likely greatly exceeded the twentieth century standards. The research by Somervaille et al. (1988) suggested also that blood lead levels are more affected by the magnitude of lead exposure, while bone lead levels are affected by both the magnitude and duration of lead exposure. Thus, the investigators found that age is a confounding variable in the relationship between blood and bone lead levels. If the correlation of bone lead levels and blood lead levels is complex in a population whose blood lead levels have been monitored over time and whose years of lead exposure and age are known, as is the case of studies of occupationally exposed lead workers (Ahlgren et al. 1980; Somervaille 1988), then correlation of bone lead levels and blood lead levels is even more precarious for an historical population whose duration of exposure to lead is unknown and whose age at death is estimated. For the individuals buried at the RNHC, it is impossible to estimate the number of years that they were exposed to lead. During the colonial era, they were undoubtedly always exposed to some lead, even in childhood. However, if rum were indeed a major contributing factor to lead exposure, then the Europeans might have been primarily exposed during their time serving in the Navy when rum was provided daily through naval rations. Therefore, as previously noted, by using the age at death as the duration of exposure for the individuals at the RNHC, it is likely that the blood lead levels have been underestimated since they are reflections of an average over the lifetime of the individual.

To further complicate the establishment of a formula to relate blood and bone lead levels, Somervaille et al. (1988) had to create a time-integrated blood lead index which took into account the blood lead levels of each of the workers over the entire period of their exposure to lead. This time integrated blood index was then compared to a single measurement of bone lead from the tibia. This, in and of itself is problematic because it would be impossible to show how bone lead levels and blood lead levels relate over the duration of exposure. The authors were thus limited to how the long-term bone lead level of the tibia can be associated with multiple measurements of blood lead over a long period of time. They were also unable to determine how the release of lead from bone into blood might have had an effect on the association between bone and blood lead levels. Despite these limitations, the investigators found that the relationship between bone and blood lead levels tended to be linear with a correlation of $R = 0.82$ for individuals who were occupationally exposed to lead. However, there was a weak correlation between blood and bone lead levels among the individuals tested who were not occupationally

exposed to lead, but rather environmentally exposed. Thus, it may be that different routes of exposure to lead may have an effect on the correlation between bone and blood lead levels. The formula derived to convert bone lead levels to blood lead levels represents an average blood lead level for the duration of exposure, which assumes a somewhat stable exposure to lead over time. Although this may be a valid assumption for occupationally exposed workers in a regulated environment, for the individuals at the RNHC, it is probable that there was more than just occupational exposure leading to bone lead levels, and that their exposure to lead was not consistent, resulting in fluctuating blood lead levels over the course of their lives. More recent research has also demonstrated that the relationship between cumulative blood lead indices (CBLI) and bone lead is not linear at higher blood lead levels due to an increase in the rate of transfer of lead from blood to bone (Behinaein et al. 2012; Healy et al. 2008). For the individuals recovered from the RNHC, several of whom had relatively high bone lead levels, this finding also suggests that the blood lead levels calculated may be underestimations. Researchers have also noted that the relationship between the CBLI and the bone lead levels is different for those who have been exposed over a long period and those who have been exposed during a shorter period (Behinaein et al. 2012; Hu et al. 2007). These new findings further complicate the estimation of blood lead levels from bone lead levels for individuals from the RNHC. Nevertheless, though a rough estimate, the conversion of bone lead levels to blood lead levels provides the opportunity to assess potential symptomatology of lead poisoning for an archaeological population, which is necessary in order to evaluate the validity of the conjecture that lead poisoning was a considerable health concern in the West Indies during the colonial period for naval personnel.

6.4 Summary

The results of the comparison between diet and bone lead levels led to an initial supposition that the strongest correlation between diet, as represented by stable isotope proxy measures, and lead was in the relationship between $\delta^{13}\text{C}_{\text{collagen}}$ and lead, followed by $\delta^{13}\text{C}_{\text{apatite}}$ and lead, and $\delta^{15}\text{N}$ and lead respectively. However, separation by ancestral groups and those of unknown ancestry revealed a more complex association that was highly variable. In the relationship between $\delta^{13}\text{C}_{\text{collagen}}$ and lead, the strongest correlation between the variables existed for those individuals consuming a diet based on C_3 staples and primarily C_3 fed terrestrial animals. For individuals consuming a mixed diet with more C_4 food contributions, the

correlation between the variables was much weaker. For the relationship between $\delta^{15}\text{N}$ and lead, the correlation was not particularly strong; however, there appeared to be a trend in which the individuals with the highest $\delta^{15}\text{N}$ values had higher bone lead levels, while the individuals with the lowest $\delta^{15}\text{N}$ values had lower bone lead levels. Despite this trend, individuals with intermediate $\delta^{15}\text{N}$ values had extremely variable bone lead levels. In the relationship between $\delta^{13}\text{C}_{\text{apatite}}$ and lead, the association between diet and lead was quite variable for both ancestries, with some individuals conforming to a pattern of higher bone lead levels coinciding with more positive $\delta^{13}\text{C}_{\text{apatite}}$ values, and others not.

A variety of challenges in the interpretation of the relationship between diet and lead arose during analysis. Firstly, there was no method to determine the source of the lead in the bones of the individuals and the extent to which this lead was a result of consumption of rum. Also, because the collection studied was a poorly historically documented archaeological population, the length of exposure to lead could not be assessed. Finally, the small sample size available for study led to some doubt as to the significance of the results, whether they were representative of a larger population or whether they were due to chance.

Estimation of blood lead levels permitted an assessment of whether individuals may have suffered symptoms of lead poisoning in life. Although a large proportion of the individuals would likely not have had symptoms of lead poisoning, there were many individuals with high blood lead levels who may indeed have had sufficient exposure to lead to cause clinical symptoms. The findings of this study are not inconsistent with the assertion by historical sources that lead poisoning was a major factor in the poor health and ineffectiveness of the British military in the West Indies.

Differences in blood lead levels between those of African and European ancestries were not statistically significant. However, it appeared that those of African ancestry may have been less likely to experience more severe symptoms of lead poisoning than those of European ancestry. Despite this difference, there is no indication that there was differential access to lead-contaminated goods based on ancestry. Rather, it appears that personal preference and social influences may have been more important in determining the extent of exposure to lead.

Challenges also arose in the estimation of blood lead levels from bone lead levels. Due to the unknown duration of exposure to lead for all the individuals at the RNHC, the estimation of blood lead levels, which takes into account the number of years that an individual has been in

contact with lead, was destined to be inaccurate and to serve only as a rough estimate. Also, the exact nature of the relationship between blood lead levels and bone lead levels for individuals who have been exposed through contaminated foodstuffs is not known and led to the necessity of using formulae designed for occupational exposure.

Findings presented in this chapter are summarized in Chapter 7, along with avenues for future investigations, and important contributions that this research has made in the field of bioarchaeology.

Chapter 7: Conclusions

7.1 Findings

The objective of this research was to examine the relationship between diet, as reconstructed via stable isotope analysis, and lead burden in the skeletal remains recovered from the Royal Naval Hospital Cemetery (RNHC). It was clear from statistical analysis and visual examination that the strongest relationship existed between $\delta^{13}\text{C}_{\text{collagen}}$ values and bone lead levels, in particular for those individuals of European and unknown ancestries. Although statistically significant correlations existed between lead and each of $\delta^{13}\text{C}_{\text{collagen}}$, $\delta^{15}\text{N}$, $\delta^{13}\text{C}_{\text{apatite}}$, none was particularly strong when individuals of all ancestral groups were tested together. The specific dietary patterns that seemed to be most correlated with increasingly higher bone lead levels for individuals of European and unknown ancestries involved consumption of C_4 fed animals and C_4 staple carbohydrates added to a primarily C_3 diet. C_4 foods would mainly have been available to individuals of European descent while they were stationed in the West Indies or other foreign postings, rather than when they lived in Europe. This suggested that, for these individuals, the more time spent in the West Indies with increased access to C_4 foods, the greater their likelihood of having somewhat higher bone lead levels. For those of African descent, with respect to their $\delta^{13}\text{C}$ values, much variability existed between the foods consumed and their bone lead levels. Thus, for individuals who likely resided in the West Indies for much or all of their lives, there was no clear indication that eating particular types of foods was associated with higher or lower bone lead levels. This may be a reflection of some of these individuals exerting their agency against the social structures of the Navy, i.e. naval rations, and excessive consumption of rum, in order to obtain more desirable or more economically beneficial goods. For all individuals, regardless of ancestry, the $\delta^{15}\text{N}$ values most correlated with higher bone lead levels were for individuals consuming larger quantities of terrestrial protein and/or locally-raised animals. Dietary patterns associated with lower bone lead levels tended to include more C_3 fed animals, C_3 staples, and less animal protein and/or more meats originating in the UK. The dietary patterns reflected general trends. However, there was much variability for individuals of all ancestral groups—particularly among those of African descent—in the relationship between diet and lead.

Blood lead levels calculated for the individuals interred at the RNHC suggested that more than half the individuals may have suffered at least mild symptoms of lead poisoning. Though mild symptoms may not have had a considerable impact on the naval personnel's capacity to carry out their duties, more severe symptoms certainly would have incapacitated an individual, especially if experienced in combination with other health concerns. Thus, the findings of the conversion of bone lead levels to blood lead levels were not inconsistent with the assertion by Buckley (1978) that lead poisoning was a substantial contributor to the downfall of the British military in the West Indies. Additionally, the calculated blood lead levels indicated that, despite suspicion by some physicians at the time that contaminated rum and other methods of lead exposure caused the symptoms of the West India dry gripes (lead poisoning), individuals in the Navy continued to receive rations of rum and may have obtained additional rum while on shore (Blane 1799; Hunter 1796; Pack 1982). Based on the blood lead levels of some of the individuals interred at the RNHC, this exposure to lead had a negative impact on their health.

There was no finding of a statistically significant difference in blood lead levels among those of different ancestral groups. Although lead exposure has been used to differentiate between ancestry and/or socioeconomic status in contemporaneous contexts (Aufderheide 1981, 1988), there was no conclusive evidence to suggest that those of European and African descent in this study had differential exposure to lead through occupation or access to contaminated goods. Some individuals from the European and unknown ancestry categories had much higher blood lead levels than those of African ancestry. However, all three categories contained individuals with very low blood lead levels, making it impossible to distinguish between African and European ancestry based on blood lead levels alone. Moreover, archaeological and historical evidence did not indicate that any apparent differences in blood lead levels were the result of differential access to goods, but conceivably as a result of personal preferences and social influences on behaviour, resulting in much variability of both bone and blood lead levels for all individuals, regardless of ancestry.

7.2 Future Areas of Research

There are many avenues along which future investigations related to, or building upon, this study might proceed. Most importantly, it would be of invaluable benefit to increase the sample size studied, if possible, through future excavations in order to determine if the patterns observed in this study are applicable to other individuals sharing a similar background, or if the

results were due to chance. The small sample size available for this study was the largest factor in casting doubt over the findings of the dietary patterns most correlated with bone lead levels. The study of more individuals interred in naval burial grounds in the West Indies would either provide further evidence for the interpretations presented in this thesis or permit the elucidation of different patterns of association between stable isotope signatures and lead burden not visible in this study.

In addition, as proposed by Farrer (1993) regarding the remains recovered from the Franklin Expedition, comparison of the individuals from the RNHC to a contemporaneous population of British civilians, should a collection become available, would demonstrate whether or not the individuals serving in the Navy were exposed to significantly more lead than the rest of the population living in Britain. Farrer (1993) suggested that individuals living in Britain in the eighteenth century were exposed to many sources of lead and must have had high background levels of lead in their bones. This assertion is not supported by the population recovered from the RNHC, in which several individuals of European descent had relatively low bone lead levels. Though in comparison to a modern Canadian population, in which bone lead levels would be expected to rise at a rate of .24 ppm/year for environmental exposure (Roy et al. 1997), the bone lead levels from the RNHC are somewhat higher. However, given the low bone lead levels for some individuals, there is evidence to suggest that not all individuals from this time period were exposed to large quantities of lead. Testing other populations from British naval cemeteries for lead exposure would also provide an additional comparative sample to determine if individuals serving in the West Indies were exposed to more lead than individuals serving elsewhere, as a result of the increased availability of rum in this region. Finally, it would be of interest to compare bone lead levels from the individuals interred at the RNHC to other archaeological findings of bone lead levels, particularly contemporaneous or geographically relevant populations, such as the enslaved labourers on the island of Barbados (Corruccini et al. 1987; Handler et al. 1986) and the sailors of the Franklin Naval Expedition (Kowal 1986; Keenleyside 1996).

Further areas of future investigations could serve to address some of the limitations of this study. One of the challenges faced was an inability to determine the duration of exposure to lead for the individuals at the RNHC. The bone samples used to investigate the relationship between diet and lead were taken from the fibula in order to test cortical bone, which has been

determined to be the best indicator of long-term lead accumulation in the body (Barry 1975; Corruccini et al. 1987; Somervaille 1988; Wittmers et al. 1988). Though this has provided adequate data for the analyses presented in this thesis, alternative information on lead exposure can be gained from testing various skeletal elements, including those consisting of primarily trabecular bone. It has been found that skeletal lead levels in an individual differ in cortical and trabecular bone (Wittmers et al. 1988). Thus, having samples of both types of bone from the same individuals might provide some additional data that would be reflective of different periods of exposure. To further investigate the concept of different types of bone reflecting different periods during an individual's life, some paleodietary studies have employed bone density fractionation to elucidate life history trajectories for individuals based on changes in diet throughout their lifetime (Bell 2001; Shin 2011). This method is based on younger or more recently formed bone being less dense than older bone. Though designed for stable isotope analysis, this method has the potential to distinguish between long-term consistent accumulation of lead in bone vs. periods of more intensive incorporation of lead into bone as a result of considerable changes in circumstance, such as joining the Navy. Investigations into the duration of exposure to lead have been conducted using synchrotron X-ray fluorescence by Martin et al. (2013) for several skeletal samples from the Franklin Expedition. They determined that exposure to lead was long-term rather than primarily due to exposure during the expedition itself. Although the study was limited, in that it could not quantitate the lead that was taken up into the bones during the crew's time on the ship, it did provide some evidence of the sequencing of lead uptake. Investigations that aim to determine the chronology of lead uptake into bone could provide a window into the extent and duration of exposure for the individuals interred at the RNHC and allow for a more precise study of the relationship between diet and lead, as well as permit a more accurate calculation of blood lead levels from bone lead levels.

Finally, studies that determine the type or source of a trace element, through isotopic analysis and chemical speciation, could be applied to the remains from the RNHC to provide additional information regarding the type of lead incorporated into bone. Chemical speciation of mercury (Hg) found in a bone sample from the RNHC has been carried out (Swanston et al. 2015). This could be applied to lead in order to better understand the form of lead to which individuals were exposed. Lead isotopic analysis, as has been carried out by Martin et al. (2013),

could provide evidence of multiple sources of lead exposure if testing revealed the presence of different lead isotope ratios in different types of bone (i.e. trabecular vs. cortical).

7.3 Important Contributions

This research has contributed to greater knowledge of the lifeways of individuals, free and enslaved, serving in the British Navy in the West Indies during the colonial period. It was the first to investigate patterns of association between diet and lead, which, given the historically proposed relationship between lead poisoning and contaminated rum in the West Indies, was a fundamental area of investigation. Potential dietary patterns associated with bone lead levels have been proposed, and have demonstrated that there was no simple relationship between stable isotope signatures and lead burden. Though there were differences in the strength of the correlation between diet, reconstructed via stable isotopes, and lead due to ancestry, this did not suggest differential access to goods, but rather an inability to see an association among the variables when the dietary signatures differed substantially among individuals. This research has provided further evidence to substantiate the argument that lead poisoning was a considerable health concern for individuals serving in the Navy in the West Indies. Finally, though it would seem that some individuals of European descent may have suffered more severe symptoms of lead poisoning, there was not sufficient evidence to suggest that this difference was based solely on ancestry. The results of this research examining both lead exposure and dietary regimes did not conform to expectations and assumptions surrounding the health and well-being of individuals of African descent in the colonial West Indies, and demonstrated that enslaved labourers owned by the Navy were likely treated quite differently from enslaved labourers who worked on plantations, and who, based on historical and archaeological evidence suffered a worse fate. In fact, this research has provided evidence that ancestry may not have been a key determinant of health for individuals serving the Navy, but agency, socio-cultural influences, and duration of service in the Navy may have been more important factors related to physical well-being.

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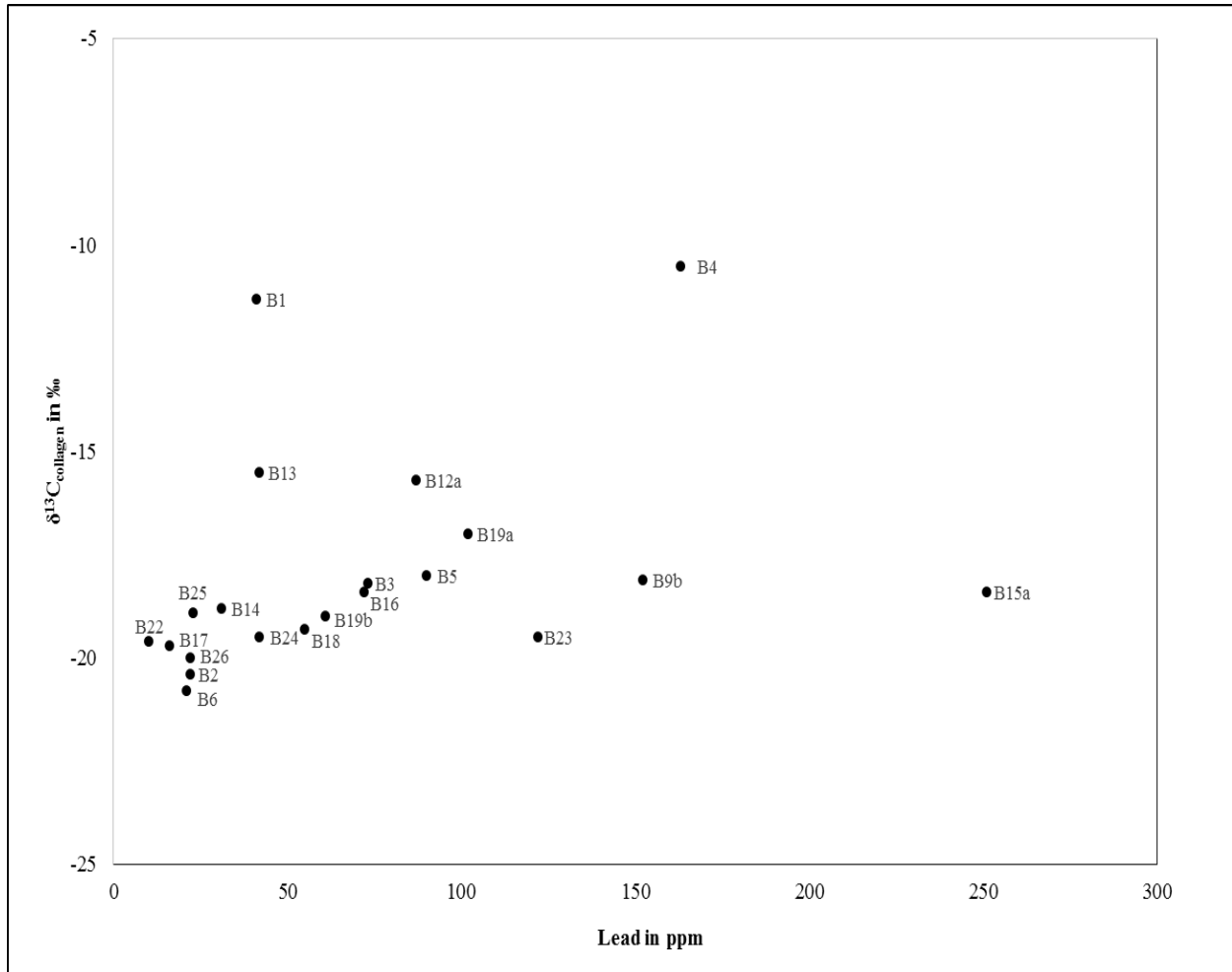
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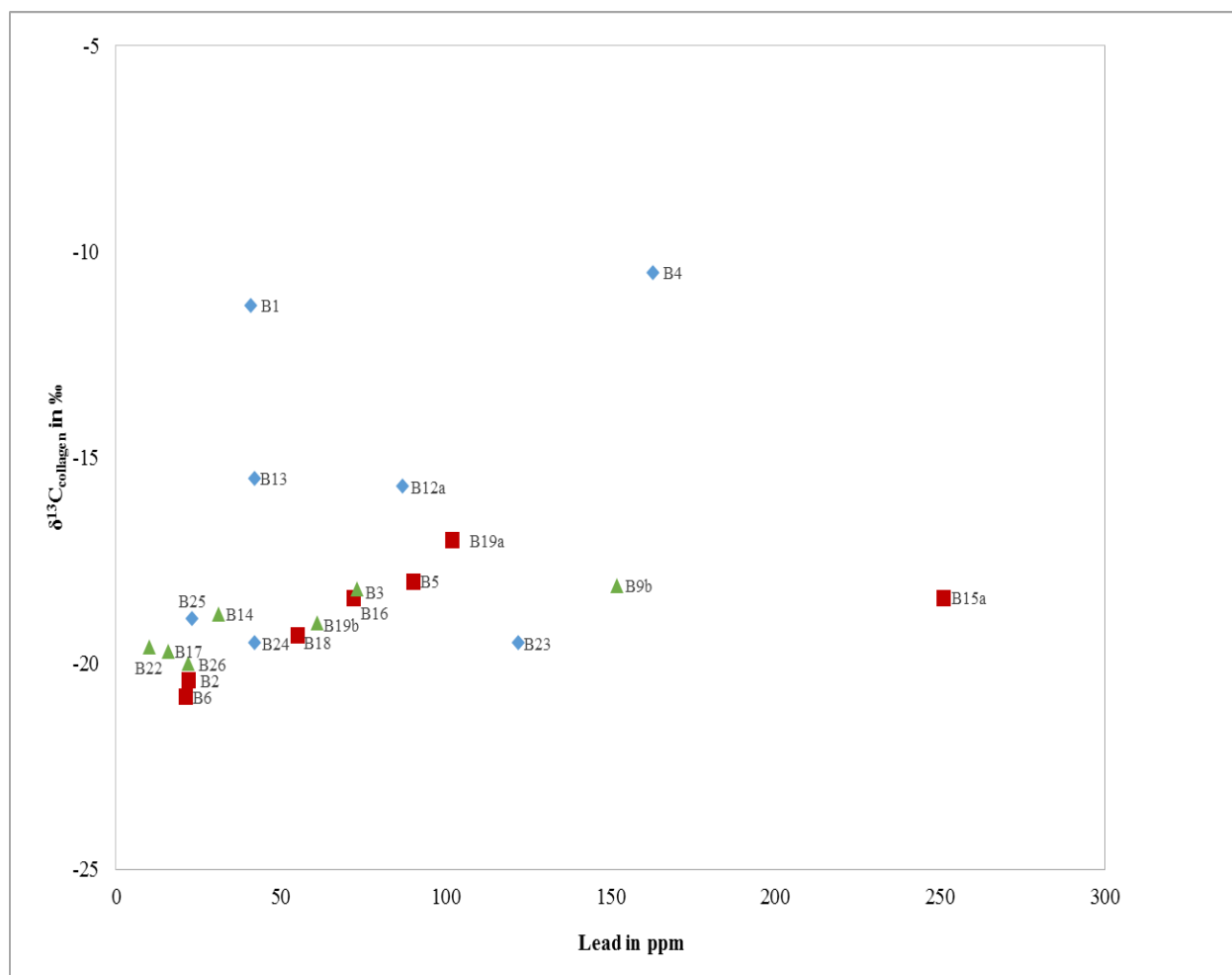
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Appendix A: Lead vs. $\delta^{13}\text{C}_{\text{collagen}}$ Labeled by Individual

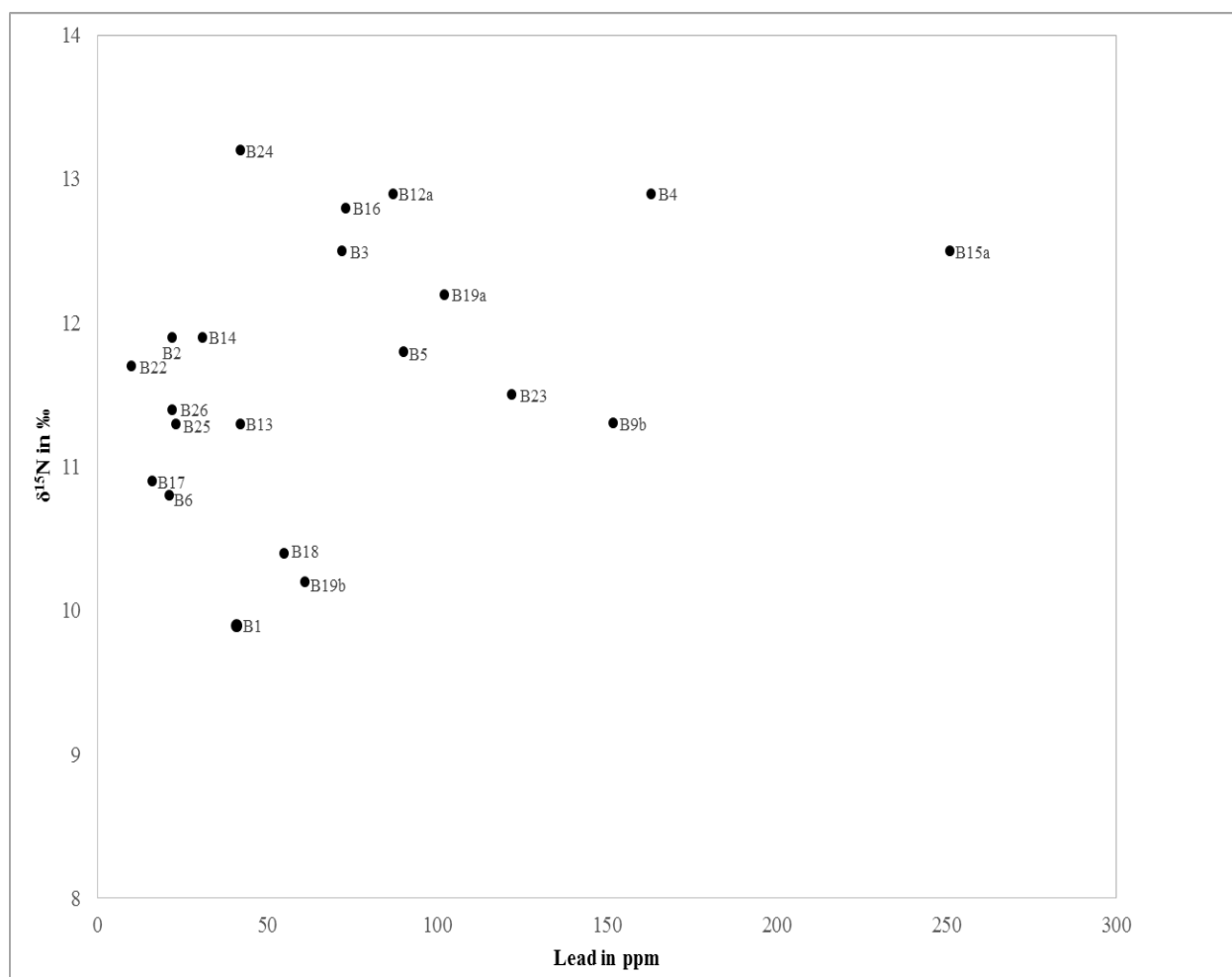


Appendix A: Lead vs. $\delta^{13}\text{C}_{\text{collagen}}$ Labeled by Individual*

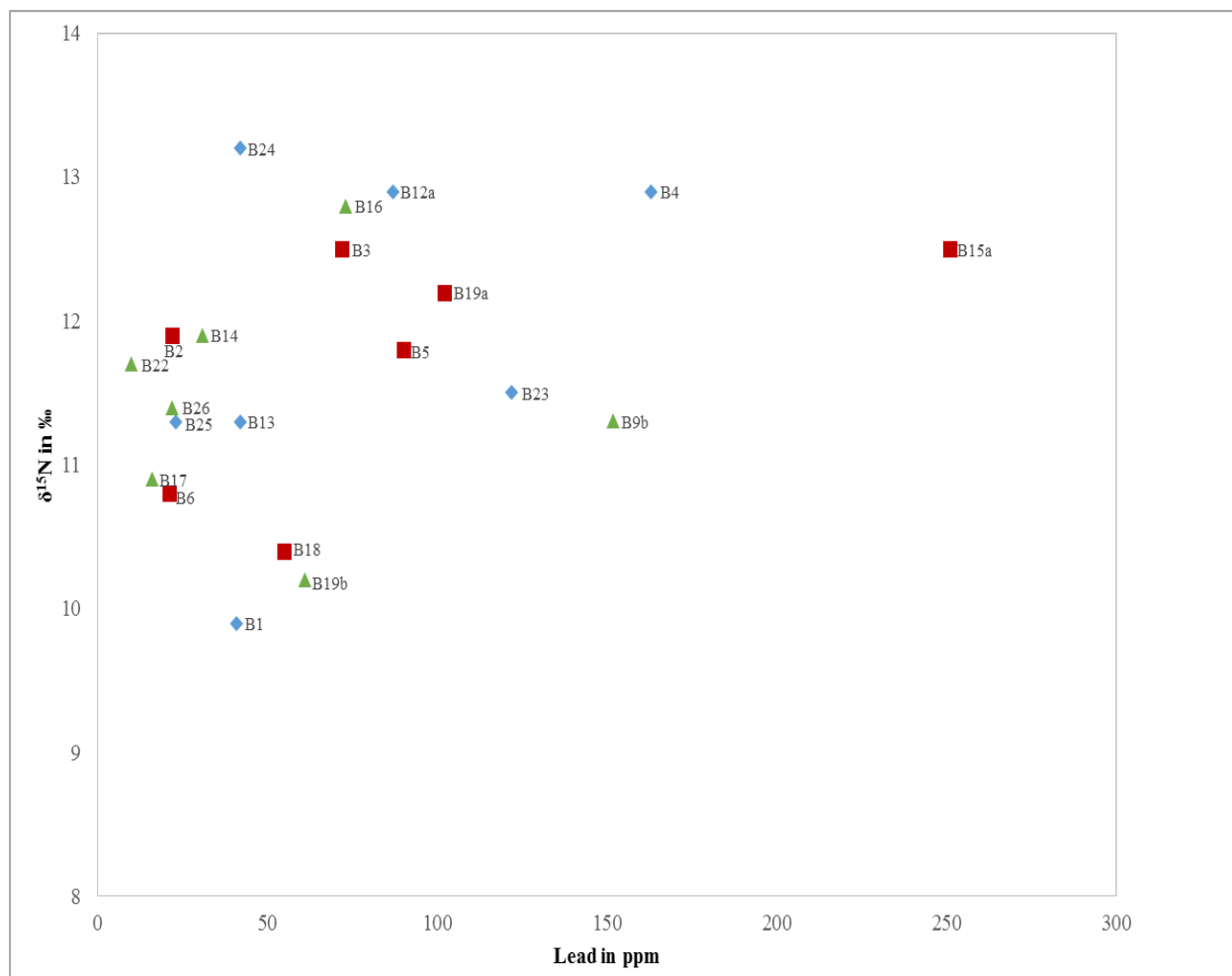


* African (◆), European (■), Unknown (▲).

Appendix B: Lead vs. $\delta^{15}\text{N}$ Labeled by Individual

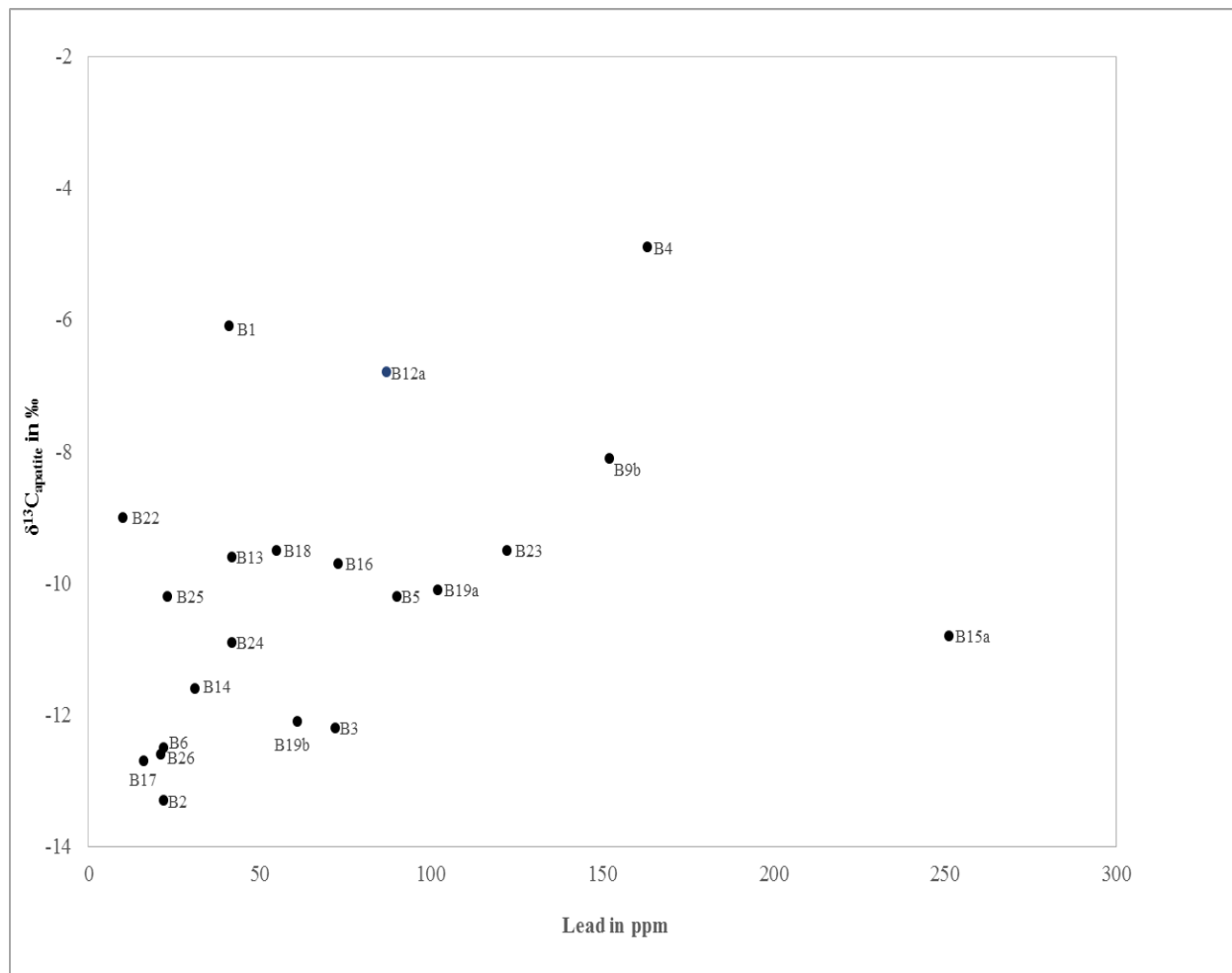


Appendix B: Lead vs. $\delta^{15}\text{N}$ Labeled by Individual*

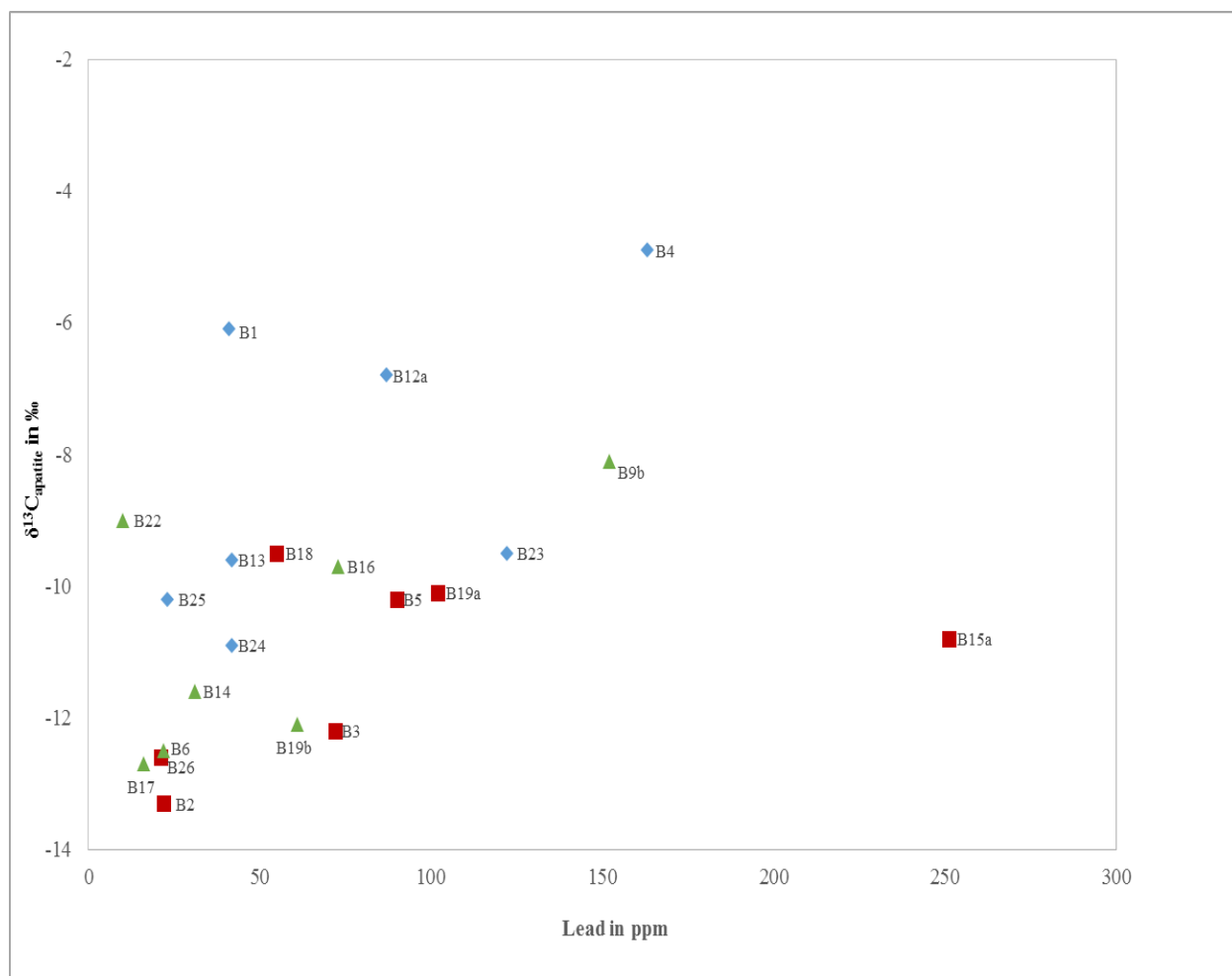


* African (◆), European (■), Unknown (▲).

Appendix C: Lead vs. $\delta^{13}\text{C}_{\text{apatite}}$ Labeled by Individual



Appendix C: Lead vs. $\delta^{13}\text{C}_{\text{apatite}}$ Labeled by Individual*



* African (◆), European (■), Unknown (▲).